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ERRATA

Page 15, line 10, "*A. Schiemanni* (Schiemann) Thom, n. comb.=*A. fuscus* Schiemann" should read "A. Schiemanni Thom, new name=*A. fuscus* Schiemann."

Page 15, line 14, "combination" should read "name."

Page 103, lines 4 and 5 from bottom, "Pl. I, fig. 7" should read "Pl. I, fig. G." Line 6 from bottom "Pl. I, fig. 6" should read "Pl. I, fig. F."

Page 128, line 3, "OH^- of 0.5×10^{-7}" should read "H^+ of 0.5×10^{-7}."

Page 254, Plate 13, last line, "Plate 12" should read "Plate 11."

Page 256, line 2 from bottom, "the salt (sodium chlorid) or sugar solution" should read "the salt or sugar solution."

Page 294, Table II, column 3, "Gratz" should read "Grah" and "*Nicotiana atropurpurea*" should read "*Nicotiana atropurpurea*[a]."

Page 382, Table IV, column 9, line 5, "3733" should read "3773." Column 10, line 7, "25.26" should read "25.36."

Page 386, line 13 from bottom, "863" should read "862."

Page 387, bottom line "1,91" should read "1,913."

Page 417, line 12, "Lipman (16)" should read "Lipman (17)."

Page 418, line 9, "Lipman (16)" should read "Lipman (17)." Lines 27 and 28, "Lipman and Burgess (17, 18, 19, 22, 23)" should read "Lipman and Burgess (16, 18, 19, 22, 23)."

Page 424, Table III, column 1, "0.75 per cent" should read "0.075 per cent."

Page 446, Table IV, column 4, "Loss in 34 days" should read "Loss in 81 days."

Pages 481 to 486, "*Nicotiana viscosum*" should read "*Nicotiana glutinosa*."

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COMPARISON OF THE NITRIFYING POWERS OF SOME HUMID AND SOME ARID SOILS[1]

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INTRODUCTION

It will be remembered by those who are interested in the subject under consideration that Stewart (11)[2] took occasion in 1912 to question the correctness of the view then prevalent, due principally to the high authority of Hilgard (3, p. 68), that nitrification proceeds with great intensity in arid soils. It will also be remembered that in the statement above cited and in subsequent papers (12, 13) Stewart controverted the theory of Headden (1, 2) with respect to the cause of unusually large nitrate accumulations in certain Colorado soils on similar grounds. The evidence presented for the latter controversion consisted of numerous analyses of irrigated and unirrigated soils at the Utah Agricultural Experiment Station and of soil and soil-forming material of the Book Cliff areas in Utah and Colorado. The analyses represented the quantity of nitrogen in the form of nitrates or the quantities of other "alkali" salts, or both, in the various soils and soil-forming materials at the time the sampling was done. As a result of his studies of these data, Stewart concluded that the intense power of nitrification attributed to arid soils, because of the large amounts of nitrates present in the field, is nonexistent, that it may actually be feeble as compared with that of humid soils, and that the large quantities of nitrates found as noted can be more readily and plausibly explained on the basis of accumulation by water movement from adjacent soil or soil-forming materials rich in salts (including nitrates). The latter contention is supported by numerous analyses of the large variety of soils, sandstones, and shales

[1] The samples of soil from the different States were sent us by the chemists or agronomists of the several Stations, and acknowledgment in addition to that already made is here publicly expressed. We owe and express our sincere thanks also to Prof. C. F. Shaw, in charge of the soil-survey work of the California Agricultural Experiment Station, for allowing us a portion of every soil sample collected iu the soil-survey areas which have already been mapped in this State.

[2] Reference is made by number to "Literature cited," p. 82.

involved, which indicate that an increase in the nitrate content of soils, such as those described by Headden (1, 2) in Colorado, is always accompanied by an increase in the quantity of the other alkali salts. Not only on the basis of his own data obtained as above but also on those of Headden himself, Stewart was able to make out a strong case in support of his explanation anent the origin of the heretofore-mysterious "niter-spots."

It will be noted that both Hilgard and Stewart employed actually or by implication an indirect method for arriving at their conclusions. While Stewart's method was less indirect in that seasonal variations in nitrate accumulations were studied, thus allowing something like a measurement of the soil's nitrifying power in the field, it can at best be productive only of results which call for further investigation. In other words, the determination of nitrates in field soils, no matter how frequently made, as a basis for determining the power of the soil to nitrify any nitrifiable material, is subject to more objections because of a lack of control of conditions than is a direct, if arbitrary, method of determining the nitrifying power of a soil in the laboratory. In an attempt, therefore, to obtain data by a more direct method, which might serve to reveal the truth about the nitrifying powers of humid and arid soils, the writers have carried out a series of studies the results of which form the principal topic of this paper.

PLAN, MATERIAL, AND METHODS OF EXPERIMENTS

In the experiments the plan was to compare under controlled and uniform conditions in the laboratory the nitrifying powers of a large number of representative soils from both humid and arid regions. In seeking for a scheme to use as the basis for the selection of such soils, it occurred to the senior author that it could be arranged by employing one soil type at least from every State in the Union to compare with a large number of California soils collected in connection with the soil-survey work of the State. Fortunately, there had just been completed a collection of soils from all the States and Territories in the Union (at least one soil and one subsoil from each State or Territory) and the writers were therefore supplied with what might be regarded as representative soil material. For the illustration of arid soils the samples collected to represent the different types of four soil-survey areas in California were employed. Approximately 45 humid were compared with 150 arid soils.

The nitrifying power of each soil was determined, using the nitrogen in the soil, in sulphate of ammonia, in dried blood, and in cottonseed meal for every soil. The last-named was employed in 100-gm. portions in tumblers, as in the methods now common among soil bacteriologists. The soils containing as nearly as possible optimum amounts of water were

incubated for one month at 28° to 30° C. The usual devices were employed for preventing an undue evaporation of moisture, the maintenance of a uniform water supply, and for mixing soil with fertilizer materials. The soil nitrogen was, of course, employed as naturally occurring in the 100-gm. soil portions, sulphate of ammonia (in solution) was employed at the rate of 0.2 per cent, dried blood at the rate of 1 per cent, and cottonseed meal at the rate of 1 per cent, all based on the air-dry weight of the soil. The phenoldisulphonic-acid method for determining nitrates, as described by Lipman and Sharp (8), was employed throughout the experiments, except as otherwise stated. The nitrate content of the original soil was subtracted in all cases, and calculations were made of the absolute amounts of nitrates produced, of the total nitrogen present (whether soil nitrogen alone or soil nitrogen plus fertilizer nitrogen), and of the percentage of the latter which was transformed in a month's incubation period into nitrates. For the purposes of the last-named determination only the complete whole numbers for the percentage concerned were computed, a plus sign being used after every one to indicate that the exact percentage was less than 1 per cent in excess of the number given.

Throughout these experiments the writers have been cognizant of the weaknesses which obtain in any method yet devised to obtain results in the laboratory with soil-bacterial activities which are directly translatable into terms of field conditions and magnitudes. For example, the fact that uniform moisture and such exceptional air conditions as are present in a constant-temperature incubator are not to be found in the field has not been overlooked. Nor yet have the writers assumed that the large amounts of fertilizer employed by them in the experiments exercise the same effect as the much smaller quantities employed in farm practice. There has been employed as a basis what seems to be the reasonable hypothesis that soils and fertilizers, particularly the former, bearing a certain relationship to one another as regards nitrifying power in the laboratory, should bear approximately the same relationship to one another in the field. This should be particularly so as regards results obtained with the soil's own nitrogen.

Further, the writers also realize two other serious difficulties which beset the investigator engaged on problems such as the one in hand. Seasonal variations in the nitrifying powers of soils are of great magnitude. This has been demonstrated by numerous investigators. Not the least disquieting data on that subject are in the hands of the writers, representing the most extensive study yet carried out on seasonal variations in the ammonifying and nitrifying powers of soils. The other difficulty is that the soils which are compared with arid soils were collected in most of the States on Experiment Station lands, which are not truly representative of average conditions obtaining among

humid soils. A minor objection which has been urged may also be mentioned here—namely, that of a given lot of soil collected at the same time two 100-gm. samples may give widely different nitrifying powers. It may be observed here that the writers have never been able to substantiate such a claim. They have merely called attention to some of the most important and perhaps the only important objections in the path of validating such comparative studies and other similar ones. It will be noted that scarcely any comment has been made regarding the objections and difficulties in question. Occasion will be taken to examine them critically and evaluate their importance in a more proper place. It may be said that in these experiments the attempt has been made to obtain relative and not absolute values, and despite the accompanying weaknesses of the methods, it is believed that current ideas with respect to the intensity of nitrification in the soils of humid and of arid regions have been improved and rendered more definite.

EXPERIMENTS WITH "FOREIGN"[1] SOILS

The word "foreign" is applied in this paper to soils coming from other States than California, unless specifically qualified in some other manner. While subsoils were available in every case and were studied in the foreign soils and while several soil types were available from some States, only one such type of surface soil and the results obtained therewith will be discussed. The results obtained with the foreign soils, arranged as above described, follow in Table I, which gives the results of nitrification of the soil, sulphate of ammonia, dried blood, and cotton-seed-meal nitrogen.

TABLE I.—*Comparison of the nitrification in soils from various States*[a]

State.	Soil type.	Soil nitrogen (Group I).			Soil nitrogen and sulphate of ammonia (Group II).			Soil nitrogen and dried blood (Group III).			Soil nitrogen and cotton-seed meal. (Group IV).		
		Nitrate nitrogen produced.	Total soil nitrogen present.	Nitrogen in soil nitrified.	Nitrate produced.	Total nitrogen present.	Nitrogen nitrified.	Nitrate produced.	Total nitrogen present.	Nitrogen nitrified.	Nitrate produced.	Total nitrogen present.	Nitrogen nitrified.
		Mg.	*Mgm.*	*Per ct.*	*Mg.*	*Mgm.*	*Per ct.*	*Mg.*	*Mgm.*	*Per cent.*	*Mg.*	*Mgm.*	*Per ct.*
Alabama	Norfolk sandy loam.	9.20	23.60	38+	1.50	63.60	2+	0.10	155.60	Tr.	4.10	73.60	5+
Alaska	Rich peat	.....	225.20	....	1.10	265.20	Tr.	81.10	357.20	22+	25.10	272.20	9+
Arizona	Fine sandy loam (Alkali).	.....	40.00	....	.....	86.00	....	.....	178.00	.....	.....	93.00	....
Arkansas	Huntington clay loam.	13.80	68.40	20+	5.40	108.40	4+	7.80	200.40	3+	13.30	115.40	11+
Colorado	Silt loam	7.50	108.30	6+	17.50	148.30	11+	29.50	240.30	12+	19.50	155.30	12+
Connecticut	Gloucester fine sandy loam.	5.60	209.80	2+	7.00	249.80	2+	9.00	341.80	2+	18.00	256.80	7+
Delaware	Sassafras loam	54.75	109.00	50+	34.75	149.00	23+	66.75	241.00	27+	54.75	156.00	35+
Florida	Calcareous peat (Everglades).	54.00	1894.00	3+	32.00	1934.00	1+	52.00	2026.00	2+	42.00	1941.00	2+

[1] This term was borrowed from Sackett (10).

[a] Does not include Hawaii, Porto Rico, or California.

TABLE I.—*Comparison of the nitrification in soils from various States*—Continued.

State.	Soil type.	Soil nitrogen (Group I).			Soil nitrogen and sulphate of ammonia (Group II).			Soil nitrogen and dried blood (Group III).			Soil nitrogen and cotton-seed meal (Group IV).		
		Nitrate nitrogen produced.	Total soil nitrogen present.	Nitrogen in soil nitrified.	Nitrate produced.	Total nitrogen present.	Nitrogen nitrified.	Nitrate produced.	Total nitrogen present.	Nitrogen nitrified.	Nitrate produced.	Total nitrogen present.	Nitrogen nitrified.
		Mg.	*Mgm.*	*Per ct.*	*Mg.*	*Mgm.*	*Per ct.*	*Mg.*	*Mgm.*	*Per cent.*	*Mg.*	*Mgm.*	*Per ct.*
Georgia	Cecil loam	9.85	27.80	35+	6.85	67.80	10+	2.85	159.80	1+	11.85	74.80	15+
Guam		18.80	177.60	10+	28.80	217.60	13+	35.80	309.60	11+	30.80	224.60	13+
Idaho	Silt loam	12.05	152.40	7+	5.25	192.40	2+	25.25	284.40	8+	18.25	199.40	9+
Illinois	Marshall silt loam	32.00	219.60	14+	4.00	259.60	1+	27.00	351.60	8+	15.00	266.60	5+
Indiana	Miami silt loam	8.42	111.80	7+	15.42	151.80	10+	33.42	243.80	13+	20.42	158.80	12+
Iowa	Wisconsin drift	17.40	146.80	11+	3.50	186.80	1+	61.50	278.80	22+	14.50	193.80	7+
Kansas	Oswego silt loam	24.30	177.60	13+	6.10	217.60	2+	30.50	309.60	9+	17.50	224.60	7+
Kentucky	Lexington limestone soil.	4.15	149.60	2+	.95	189.60	Tr.	23.95	281.60	8+	11.95	196.60	6+
Louisiana	Orangeburg sandy loam.	4.35	64.20	6+	.75	104.20	Tr.	.55	186.20	Tr.	21.95	111.20	19+
Maine	Aroostook loam	16.05	148.20	10+	11.25	188.20	5+	31.25	280.20	11+	20.25	195.20	10+
Maryland	Sassafras clay loam.	15.98	72.60	22+	7.38	112.60	6+	18.78	204.60	9+	10.78	119.60	9+
Massachusetts	Podunk fine sandy loam.	7.88	151.00	5+	12.88	191.00	6+	17.88	283.00	6+	19.88	198.00	10+
Michigan	Miami sandy loam.	17.40	112.5	15+	6.40	152.5	4+	31.40	244.50	12+	14.40	159.50	9+
Minnesota	Fargo clay loam	37.00	316.20	11+	20.00	356.20	5+	53.00	448.20	11+	18.00	363.20	4+
Mississippi	Loam	7.70	34.80	22+	4.70	74.80	6+	—.30	166.80	.....	4.70	81.80	5+
Missouri	Upland silt loam	10.15	89.40	11+	5.75	129.40	4+	26.75	221.40	12+	14.75	136.40	10+
Montana	Yakima silt loam	18.00	218.20	8+	11.50	258.20	4+	35.50	350.20	10+	18.50	265.20	6+
Nebraska	Fine sandy loam	39.30	76.80	51+	14.30	116.80	12+	.30	20.80	Trace.	20.30	123.80	16+
Nevada	Clay loam	15.00	67.00	22+	4.00	107.00	3+	22.00	199.00	11+	17.00	114.00	14+
New Hampshire	Boulder clay	13.57	211.20	6+	5.57	251.20	2+	18.57	343.20	5+	9.97	258.20	3+
New Jersey	Sassafras loam	5.68	83.80	6+	.68	123.80	Tr.	13.68	215.80	6+	11.68	130.80	8+
New Mexico	Anthony fine sandy loam.	15.00	39.00	38+	12.00	79.00	15+	-1.00	171.00	.....	9.00	86.00	10+
New York	Dunkirk clay loam.	24.85	160.80	15+	16.85	200.80	8+	39.85	392.80	14+	22.85	207.80	10+
North Carolina	Cecil sandy loam	12.52	40.40	30+	2.87	80.40	3+	19.87	172.40	11+	14.87	87.40	17+
North Dakota	Clay loam	6.20	279.80	3+	12.00	319.80	3+	42.00	411.80	10+	17.00	326.80	5+
Ohio	Wooster silt loam	23.70	120.20	19+	3.70	160.20	2+	26.70	252.20	10+	14.70	167.20	8+
Oklahoma	Loam	6.75	82.40	8+	2.75	122.40	2+	12.75	214.40	5+	11.75	129.40	9+
Oregon	Willamette Valley sandy loam.	13.20	935.00	1+	47.20	975.00	4+	57.20	1067.00	5+	50.20	982.00	5+
Pennsylvania	Hagerstown silt loam.	19.00	127.20	14+	7.00	167.20	4+	26.00	259.20	10+	17.00	174.20	9+
Rhode Island	Miami silt loam	8.55	163.60	5+	3.25	203.60	1+	19.25	295.60	6++	12.25	210.60	5+
South Carolina	Cecil sandy loam	13.20	43.20	30+	11.00	83.20	12+	—.20	175.20	.....	19.00	90.20	21+
South Dakota	Clay loam	19.60	193.00	10+	13.60	233.00	5+	29.60	325.00	9+	22.60	240.00	9+
Tennessee	Upland loam	11.06	102.00	10+	4.26	142.00	3	21.86	234.00	9+	14.86	149.00	9+
Texas	Black adobe	15.40	103.40	14+	24.40	143.40	17+	49.40	235.40	20+	19.40	150.40	12+
Utah	Greenville loam	22.90	128.60	17+	28.90	168.60	17+	48.90	260.60	18+	24.90	175.60	14+
Vermont	Sandy loam	8.80	85.20	10+	2.80	125.20	2+	25.80	217.20	11+	14.80	132.20	11+
Virginia	Hagerstown loam	15.20	95.00	16+	6.00	135.00	4+	27.00	227.00	11+	18.00	142.00	12+
Washington	Shot clay	6.60	41.80	15+	2.60	87.80	2+	35.80	173.80	20+	9.00	94.80	9+
West Virginia	Meigs sandy loam	7.84	184.60	4+	1.44	224.60	Tr.	9.84	316.60	3+	6.04	231.60	2+
Wisconsin	Carrington sandy loam.	16.50	174.80	9+	11.50	214.80	5+	28.50	306.80	9+	22.50	221.80	10+
Wyoming	Loam	15.15	99.20	15+	24.15	139.20	17+	57.15	231.20	24+	27.15	146.20	18+

Before discussing nitrification proper it is of importance to note the conditions which obtain in the foreign soils with respect to total nitrogen content. This consideration is important because it emphasizes what has been only vaguely appreciated in the past—namely, the great discrepancy between the nitrogen content of humid and of arid soils. Moreover, it is important because the soil's total nitrogen content may have a bearing on the absolute quantities of nitrates produced through the

agency of nitrification. Out of 49 foreign soils, about 9 of which should be reckoned as arid rather than humid, 30, or about 61 per cent of the whole number, contain more than 0.1 per cent of total nitrogen (only in very small part, including nitrates). If allowances are made for the arid or semiarid soils among the foreign soils, about three-quarters of the soils from the humid region are found to contain more than 0.1 per cent of nitrogen. Only 8 soils, or about 14 per cent of the total of 49, contain less than 0.05 per cent of nitrogen each, and 4 of these belong properly to the arid or semiarid class. Only 2 soils contain less than 0.03 per cent total nitrogen, but neither of these is an arid soil. These figures are very interesting and worth remembering for comparison later with similar statistics anent the California soils. The second column of figures in Group I of Table I can be made to show percentages of total nitrogen by moving the decimal point three places to the left.

With respect to the absolute quantities of nitrate produced, only 16 out of 47 soils tested produced from their own nitrogen less than 10 mgm. of nitrate nitrogen, and only 2 of them produced less than 5 mgm. of nitrate nitrogen in the same period. No relationship whatever is discernible between the total amount of nitrogen present in the soil and the amount which was rendered into nitrate. About two-thirds of the soils tested were therefore able to produce in every case more than 10 mgm. of nitrate nitrogen in a month's incubation period. Likewise, nearly two-thirds of the soils tested rendered more than 10 per cent of the nitrogen present in the soil into nitrate, and several more approached the 10 per cent mark very closely. Moreover, nearly 40 per cent of the soils tested transformed in every case more than 15 per cent of the total nitrogen present into nitrate. If the few characteristically arid or semiarid soils among the foreign soils are disregarded, less than 35 per cent of the soils would fall in the class last named. It is also of great interest to note that over 12 per cent of the soils tested transformed more than 30 per cent of the nitrogen in them into nitrate under the circumstances noted; and two of the soils, the Delaware and Nebraska samples, transformed into nitrate more than 50 per cent of the nitrogen which they contain. It may be purely a matter of coincidental interest but possibly worthy of note that of the 9 soils which transformed between 20+ per cent and 38+ per cent of their nitrogen into nitrate, thus placing them in a class next to the two very exceptional soils just referred to, 7 belong to the Southern or South Atlantic group of States, and the other 2 are from New Mexico and Nevada, which more properly belong with the arid or semiarid group.

The results obtained when sulphate of ammonia is added to the soil nitrogen and the whole incubated are found to be opposite to that taken by the data for soil nitrogen. The addition of sulphate of ammonia to the foreign soil has not induced, as might be expected, an increase in the production of nitrate over that produced from the soil's nitrogen alone,

but has, on the contrary, caused a reduction in the soil's power to render nitrogen into nitrate. This holds, of course, only in general, and several exceptions may be found to the rule. If the absolute amounts of nitrate produced as given in Group II of Table I are compared specifically with those given in Group I, it will be found that all the soils tested except six—namely, those from Indiana, Oregon, Texas, Utah, Wyoming, and Guam—produced far less nitrate when sulphate of ammonia was added to them than they did from their own nitrogen alone. It will be noted that of the six soils which did produce more nitrate under the conditions noted than from their own nitrogen only one is a soil belonging strictly to the humid region. The effects of sulphate of ammonia on the nitrification of soil nitrogen in humid soils is very striking and difficult to explain. The acidity of soils appears to be inadequate to explain the situation.

As is to be expected, smaller absolute transformations of nitrogen into nitrate in soil and ammonium sulphate than in soil nitrogen mean a smaller percentage transformation of the total nitrogen present. Hence, whereas in the case of the soil nitrogen alone, 11 soils out of 47, or 23 per cent, transformed more than 20 per cent of nitrogen in every case into nitrate, only one soil belongs in that class when the series containing soil nitrogen plus sulphate of ammonia nitrogen is considered. Also, whereas nearly 66 per cent of the soils in the soil-nitrogen group transform in every case more than 10 per cent of the total nitrogen into nitrate, only about 23 per cent of the same soils in the ammonium-sulphate group fall in that category. These limited statistical illustrations on the differences obtaining between the experimental series resulting in the soil-nitrogen group and the ammonium-sulphate group are sufficiently emphatic to need no further comment at this point.

Group I of Table I is compared with Group III, which sets forth the results of the dried-blood series with the foreign soils, only 10 soils out of 47 or 48, or about 20 per cent, will be found which produced less nitrate when blood (1 per cent) was added to them than when only their own nitrogen supply was allowed to nitrify. Again, attention is called to what is probably a purely coincidental but interesting circumstance like the one above mentioned. Just as 9 of the soils which transformed more than 20 per cent and less than 39 per cent of their own nitrogen into nitrate included 7 which came from States of the Southern or South Atlantic group, so in this case of the 10 soils which produced less nitrate from dried-blood nitrogen plus the soil nitrogen than from the latter alone, 7 belong to the group of States which are in nearly all cases the same. Of the three other soils, two are from the semiarid region—namely, Nebraska and New Mexico—and only one is from the northern portion of the humid region—namely, Illinois. It is worthy of note that in all but the Florida and Illinois soil, of the group of 10 just considered, the nitrogen content is below 0.08 per cent, and in most of them is below 0.05 per cent.

The amounts of nitrate produced are smaller in Group III of Table I than in Group I for reasons which are obvious and which have been referred to previously. Nevertheless, in spite of the disadvantage which the added dried-blood nitrogen creates in this series with respect to the relative considerations, about 23 soils, or nearly 50 per cent of the whole number, transform more than 10 per cent of the total soil plus dried-blood nitrogen into nitrate, and several other soils approach that record closely. In five cases more than 20 per cent of the total nitrogen present is transformed into nitrate. Taken as a whole, therefore, and in spite of the large quantities of blood used, the foreign soils must be adjudged efficient nitrifiers of dried-blood nitrogen. This is particularly to be kept in mind for comparison with data from the California soils.

Group IV of Table I, which gives the results obtained in the cottonseed-meal series, is not strikingly different from Group III, which represents the dried-blood series. Nevertheless some distinct points of dissimilarity between the two require some comment. Thus, it must be noted that in the dried-blood series, 34 out of 48 soils produced more nitrate nitrogen than they did with the soil nitrogen alone. In the cottonseed-meal series only 26 out of the same total of soils accomplished that task. On the other hand, certain soils which induced only losses of nitrate nitrogen with dried blood, like the Georgia, Louisiana, and South Carolina soils, gave with cottonseed meal increases of nitrate over those produced with the soil nitrogen alone.

The percentage of total nitrogen which is transformed into nitrate in the cottonseed-meal series with the foreign soils is not strikingly unlike that of the dried-blood series when the soils are regarded as a whole. Nevertheless the individual soils show marked differences in the direction noted. Thus, for example, eight of the soils in the dried-blood series transform nothing or less than 2 per cent of the total nitrogen present into nitrate, whereas in the cottonseed-meal series no soil is productive of no nitrate, and only two fall in the 2 per cent class, or thereabouts. In other words, it would appear that while the dried blood is better suited to the foreign soils if a few soils are eliminated from consideration, cottonseed meal is better suited to the average soil, provided the influence of the amount of fertilizer used is disregarded. In general, it would appear that dried blood is a more readily and more efficiently nitrifiable material for the soils of the humid region than cottonseed meal.

In a general survey of the results obtained with the foreign soils, it seems to be true beyond question that with respect to relative quantities of nitrates produced from the different forms of nitrogen, the soil nitrogen is the most efficiently nitrified of the four forms tried. Sulphate of ammonia is the least efficiently nitrified, while dried blood and cottonseed meal differ very little. Table II summarizes the situation with respect to one degree of nitrate formation only.

TABLE II.—*Total nitrogen present transformed into nitrate in foreign soils*

Nitrifiable material.	Soils transforming 10 per cent or more of total nitrogen into nitrate.
	Per cent.
Soil nitrogen alone	68
Soil nitrogen plus ammonium sulphate	23
Soil nitrogen plus dried blood	47
Soil nitrogen plus cottonseed meal	45

Based on the absolute criterion of the production of 20 mgm. of nitrate nitrogen, or more, under the circumstances noted, sulphate of ammonia still remains the lowest in the scale with only 8 soils possessing such a record, the soil nitrogen is next with 9 soils in that class, cottonseed-meal nitrogen is next with 13 such soils, and dried-blood nitrogen stands best, with 31 such soils. The corresponding figures obtained when the production of 15 mgm. of nitrate is taken as a criterion are as follows: Soil nitrogen, 23 soils; ammonium-sulphate nitrogen, 11 soils; dried-blood nitrogen, 36 soils; and cottonseed-meal nitrogen, 28 soils.

In brief, therefore, dried-blood nitrogen takes first place for the absolute amount of nitrate produced therefrom by the foreign soils, cottonseed-meal nitrogen being second, soil nitrogen third, and ammonium-sulphate nitrogen last. On the relative basis, however, the dried-blood nitrogen goes from first to second place, and the soil nitrogen from third to first place, with the sulphate of ammonia remaining last, and the cottonseed meal third in order.

EXPERIMENTS WITH CALIFORNIA SOILS

In order to make the choice of California soils representative not only of the arid region but also of parts of the State with widely varying climatic and other conditions, soil types were chosen from two central to northwest soil-survey areas and from two southern California areas. These areas were the Ukiah and the Bay areas and the Riverside and the Pasadena areas. The rainfall for the first two areas varies from 20 to 40 inches or more a year, depending on the location; and nearly all of it falls during the winter. The precipitation for the Riverside and Pasadena areas varies from 7 to 12 inches or more, also limited almost entirely to the winter months. The nitrification tests were arranged as described above and in the same way as those of the foreign soils. The first area to be considered here is the Bay area, and Table III sets forth the results obtained with the soil types of that area and with the different forms of nitrogen.

TABLE III.—*Nitrification in types from the Bay area series*

Soil type.	Soil nitrogen (Group I).			Soil nitrogen and sulphate of ammonia (Group II).			Soil nitrogen and dried blood (Group III).			Soil nitrogen and cottonseed meal (Group IV).		
	Nitrate produced.	Total nitrogen in soil.	Nitrogen nitrified.	Nitrate produced.	Total nitrogen in soil.	Nitrogen nitrified.	Nitrate produced.	Total nitrogen in soil.	Nitrogen nitrified.	Nitrate produced.	Total nitrogen in soil.	Nitrogen nitrified.
	Mgm.	*Mgm.*	*P. ct.*	*Mgm.*	*Mgm.*	*P. ct.*	*Mgm.*	*Mgm.*	*P. ct.*	*Mgm.*	*Mgm.*	*P. ct.*
Tidal marsh clay	0.10	137.20	Tr.	2.00	177.20	1+	1.50	269.20	Tr.	5.00	184.20	2+
Altamont heavy loam	2.35	112.00	2+	7.75	152.00	5+	14.75	244.00	6+	9.75	159.00	6+
Yolo silty clay loam	1.20	93.80	1+	.80	133.80	Tr.	.60	225.80	Tr.	.45	140.80	Tr.
Dublin clay adobe	1.50	65.80	2+	4.00	105.80	3+	4.00	197.80	2+	6.00	112.80	5+
Dune sand	.80	35.00	2+	.72	75.00	Tr.	.....	167.00	.....	1.20	82.00	1+
Oakley light sandy loam	3.00	51.80	5+	4.00	91.80	4+	−1.00	183.80	.....	3.50	98.80	3+
Altamont clay adobe	.30	50.40	Tr.	.40	90.40	Tr.	.....	182.40	.....	.....	97.40	.....
Diablo clay adobe	2.00	65.80	3+	12.00	105.80	1+	7.80	197.80	3+	8.00	112.80	7+
Residual clay adobe	3.00	116.20	2+	3.00	156.20	1+	3.00	248.20	1+	2.50	163.20	1+
Santa Rosa loam	.90	86.80	1+	4.50	126.80	3+	9.50	218.80	4+	7.50	133.80	5+
Altamont light types	3.00	72.80	4+	3.00	112.80	2+	.....	204.80	.....	7.00	119.80	5+
No. 150 Brown type heavy loam	5.50	183.40	2+	20.90	223.40	9+	18.50	315.40	5+	11.50	230.40	4+
Tuscan stony loam	.43	32.20	1+	.43	72.20	Tr.	2.13	164.20	1+	.63	79.20	Tr.
Do	1.40	138.60	1+	5.30	178.60	2+	4.80	270.60	1+	4.30	185.60	2+
Santa Rosa loam	1.57	61.60	2+	1.28	101.60	1+	−.12	193.60	.....	.48	108.60	Tr.
No. 150 Gray phase loams	1.80	82.60	2+	1.80	122.60	1+	.60	214.60	Tr.	5.10	129.60	3+
Newark loam	4.10	75.60	5+	4.30	115.60	3+	25.90	207.60	12+	6.10	122.60	4+
Antioch loams and clay loams	4.36	72.80	5+	6.56	112.80	5+	15.96	204.80	7+	16.96	119.80	14+
No. 12 black phase	1.60	99.40	1+	6.40	139.40	4+	12.00	231.40	5+	7.60	146.40	5+
Auburn clay loam	.80	32.20	2+	.50	72.20	Tr.	1.00	164.20	Tr.	.10	79.20	Tr.
Corning loam	.95	44.80	2+	9.50	84.80	11+	−.55	176.80	.....	3.45	91.80	3+
Pleasanton	4.00	64.40	6+	2.80	104.40	2+	.30	196.40	Tr.	20.00	111.40	17+
Montezuma clay loam	.20	32.20	Tr.	.30	72.20	Tr.	.....	166.20	.....	.....	79.20	.....
Montezuma clay adobe	2.20	86.80	2+	8.20	126.80	6+	25.20	218.80	11+	16.20	133.80	12+
Yolo clay	3.70	113.40	3+	8.10	153.40	5+	.10	245.40	.....	1.20	160.40	Tr.
Yolo light type silt loam	4.00	131.60	3+	18.20	171.60	10+	19.20	263.60	7+	23.20	178.60	12+
Corning loam	2.44	58.80	4+	2.54	98.80	2+	−.06	190.80	.....	5.94	105.80	5+
Yolo gravelly loam	2.18	82.60	2+	4.68	122.60	3+	33.68	214.60	15+	23.68	129.60	18+
Antioch loam	.50	64.40	Tr.	.50	104.40	Tr.	.....	196.40	.....	.40	111.40	Tr.
Antioch light sandy loam	2.84	105.00	2+	14.04	145.00	9+	12.04	237.00	5+	21.04	152.00	13+

BAY AREA SOILS

The figures for total nitrogen in the second column of Table III show that only 8 soils out of 30, or approximately 26 per cent, contain more than 0.1 per cent of nitrogen, and that only one of them contains more than 0.14 per cent of nitrogen. In other words, the foreign soils contain relatively 2½ times as many soils which contain nitrogen in excess of 0.1 per cent as do the soils of the Bay area; and, moreover, many of the first-named group contain very much more nitrogen than 0.15 per cent. The effects of the arid climate are therefore quite evident on soils of the Bay area and are only emphasized by comparison with the foreign soils existing under a humid climate. It must be further remarked that the comparison gains in significance from the reflection that while the Bay area soils are subjected to a long season of drought, they receive annually between 20 and 30 inches of rainfall, depending on the part of the area concerned, and are in addition protected from excessive oxidation influences by much fog and cool weather. In respect to the number of soils

in the Bay area containing less than 0.05 per cent of total nitrogen, the last-named group of soils is not unlike the foreign group. The soils of the latter contain 14 per cent of such soils as against 16 per cent for the Bay area. No soil in the Bay area contains less than 0.023 per cent of total nitrogen.

After a study of one form of nitrogen at a time and in the same order as before, a striking difference is found between the absolute quantities of nitrates produced by the soils of the Bay area out of their own nitrogen and those produced by the foreign soils under similar circumstances. Thus, for example, only 1 soil out of 30 in the Bay area produces a little more than 5 mgm. of nitrate nitrogen, and all other soils produce less. If this observation is compared with the corresponding one for the foreign soils of the humid region, the feeble nature of the nitrifying power of the soils of the Bay area for their own nitrogen is very noticeable, as is the very vigorous power in that direction possessed by the foreign soils. In the latter only 2 soils out of a total of 47 produced less than 5 mgm. of nitrate nitrogen, while in the former only 1 soil produced more than 5 mgm. of nitrate nitrogen. The absolute magnitude of nitrate production in soils of the Bay area varies from 0.1 mgm. to 5.50 mgm., thus making a very small range. Again, there is evidence in Table III that the magnitude of nitrifying power for soil nitrogen is independent of the total amount of nitrogen present. Thus, for example, the largest production of nitrate occurs in the soil with the highest total nitrogen content in the whole series. On the other hand, the lowest nitrate production occurs in the soil with the third highest quantity of total nitrogen.

It follows from what has been said in the preceding paragraph that only small percentages of the nitrogen present in the soils of the Bay area could have been transformed into nitrate. The best record made consists in a conversion by the Pleasanton soil of over 6 per cent of the total nitrogen present into nitrates. In three other soils 5 per cent of the nitrogen was thus converted, and in the other 26 soils the records are much poorer. Comparing the relative data of Table III with those set forth in Table I, section 1, one can not help being struck by the remarkably high nitrifying efficiency of the foreign soils as compared with that of the Bay area soils.

The use of sulphate of ammonia as a nitrifiable material with the Bay area soils shows also the relatively low nitrifying power of the latter. Nevertheless Table III, Group II, brings out a very important fact—that is, that in the case of the absolute amounts of nitrate produced the Bay area soils gave increases over the amount of nitrate produced from the soil alone in all but 5 out of a total of 30 soils. This is a diametrically opposite effect to that induced by sulphate of ammonia in the foreign soils. On a relative basis the results in the sulphate-of-ammonia

series were superior to those in the soil-nitrogen series as is shown clearly in Table III, Group II, whereas in Table III, Group I, the highest percentage of nitrogen transformed into nitrate was slightly in excess of 6. The corresponding figure for Group II is 11, and several other soils, besides, approach that record. In general, it would seem that sulphate of ammonia stimulates nitrification in soils of the Bay area, while it depresses nitrification in the foreign soils.

With dried blood the Bay area soils show a loss in nitrate production over that of which the soil is capable on its own nitrogen supply in over 43 per cent of the soil types, so far as absolute quantities of nitrates are concerned. In a number of these cases, moreover, there is not only less nitrate produced than from soil nitrogen alone, but the soil's original nitrate supply is lost besides. On the other hand, there are about 9 soils in the Bay area series which, on the basis of production of nitrate in the absolute sense, surpass any in the series with soil nitrogen alone, and, similarly, all but one in the sulphate-of-ammonia series. It may be noted, however, that none of these soils consists of coarse, sandy material with a small absorbent surface, and none contains less than 0.073 per cent of total nitrogen, nearly all of them containing considerably more than 0.08 per cent. It is the last group of 9 soils showing naturally a number of good results on the relative basis in Table III, Group III, which reveals the highest percentage transformation of total nitrogen into nitrate yet noted with the Bay area soils in these experiments. The record is a 15 per cent transformation in the case of the Yolo gravelly loam. One transformation of 12 per cent and one of 11 per cent are also noted, but all the rest are considerably below those figures. In general, it would seem that the heavier soils and such as are better supplied with nitrogen than the average of the Bay area series will give better results with dried blood than with sulphate of ammonia or soil nitrogen, but the rest (more than two-thirds of the total number) will not do as well with dried blood as with the other forms of nitrogen.

Considering the cottonseed-meal results in the case of the Bay area soils, as set forth in Table III, Group IV, from the absolute amounts of nitrate produced cottonseed meal is to be regarded as of less value than dried blood from some points of view and of more value from others. To be more specific, no soil produces as much nitrate from cottonseed meal as does the Yolo gravelly loam from dried blood. On the other hand, there are less soils in the cottonseed-meal series than in the dried-blood series which are induced to lose in nitrifying power by the incorporation of the fertilizer and none at all which lose part or all of the nitrate nitrogen originally contained in them. Relatively, the cottonseed-meal series is ahead of all others with the Bay area soils in that the largest amount of nitrogen transformed into nitrate is there noted. This record is attained by the same soil as that having the record in the dried-blood series and amounts to an 18 per cent transformation of the total nitrogen present into nitrate. The Pleasanton

soil accomplishes a 17 per cent transformation in this series, and three other soils transform more than 12 per cent of the nitrogen present into nitrate. The other soils are considerably inferior in the direction noted.

PASADENA AREA SOILS

The soil types of the Pasadena area number 33, and data are here given either for the whole number or for one or two less, in accordance with the circumstances attending the experimental work. Table IV sets forth the results obtained with the Pasadena area soils with the different forms of nitrogen as above described.

TABLE IV.—*Nitrification in Pasadena area soil type*

Name of type.	Soil nitrogen (Group I).			Soil nitrogen and sulphate of ammonia (Group II).			Soil nitrogen and dried blood (Group III).			Soil nitrogen and cotton-seed meal (Group IV).		
	Nitrate produced.	Total nitrogen present in soil.	Nitrogen nitrified.	Nitrate produced.	Total nitrogen present in soil.	Nitrogen nitrified.	Nitrate produced.	Total nitrogen present in soil.	Nitrogen nitrified.	Nitrate produced.	Total nitrogen present in soil.	Nitrogen nitrified.
	Mgm.	*Mgm*	*P. ct.*	*Mgm.*	*Mgm.*	*P. ct.*	*Mgm.*	*Mgm.*	*P. ct.*	*Mgm*	*Mgm.*	*P. ct.*
Dublin clay adobe	7.60	109.20	6+	39.60	149.20	26+	2.60	241.20	1+	50.60	156.20	32+
Diablo clay	5.68	54.60	10+	15.88	94.60	16+	— .32	186.60	.....	7.68	101.60	7+
Zelzah light loam	20.30	77.00	26+	8.80	117.00	7+	— .20	209.00	.....	5.80	124.00	4+
Altamont clay	9.80	154.00	6+	47.80	194.00	24+	39.80	286.00	13+	27.80	201.00	13+
Zelzah gravelly loam	9.44	61.60	15+	15.44	101.60	15+	— .56	193.60	.....	20.44	108.60	18+
Holland loam	17.93	67.20	26+	7.93	107.20	7+	— .08	199.20	.....	27.93	114.20	24+
Hanford coarse sandy loam	4.28	54.60	7+	9.38	94.60	9+	— .13	186.60	.....	.68	101.60	Trace
Hanford gravelly sandy loam	5.59	68.60	8+	17.19	108.60	15+	— .81	200.60	.....	— .81	115.60	.....
Hanford sandy loam	3.70	40.60	9+	5.90	80.60	7+	— .30	172.60	.....	3.90	87.60	4+
Hanford fine sandy loam	.....	.....	.....	15.57	.....	.....	— .43	.....	.....	13.58	.....	.....
Hanford loam	.....	100.80	.....	14.79	140.80	10+	9.79	232.80	4+	39.79	147.80	26+
Tejunga stony sand	4.00	18.20	21+	2.80	58.20	4+	.....	150.20	.....	.....	65.20	.....
Zelzah stony loam	6.00	64.40	9+	7.00	104.40	6+	.....	196.40	.....	24.00	111.40	21+
Hanford stony loam	3.88	28.00	13+	6.28	68.00	9+	— .13	160.00	.....	— .13	75.00	.....
Placentia loam	5.50	68.60	8+	3.00	108.60	2+	— 1.50	170.60	.....	28.50	115.60	24+
Holland sandy loam	5.39	46.20	11+	3.39	86.20	3+	— .61	178.20	.....	5.39	93.20	5+
Hanford stony sandy loam	3.80	67.20	5+	9.30	107.20	8+	— 2.20	199.20	.....	11.80	114.20	10+
Antioch clay loam	8.00	182.00	4+	38.00	222.00	17+	122.00	314.00	38+	36.00	229.00	15+
Chino silty clay loam	14.40	151.20	9+	42.40	191.20	22+	79.40	283.20	27+	30.40	198.20	15+
Chino clay adobe	11.00	382.20	2+	61.00	422.20	14+	119.00	514.20	23+	33.00	429.20	7+
Chino loam	— 9.00	187.60	.....	10.00	227.60	4+	36.00	319.60	11+	68.00	234.60	28+
Hanford fine sandy loam	13.20	88.20	14+	45.20	128.20	35+	8.70	220.20	3+	77.20	135.20	57+
Chino silty clay loam	7.00	219.80	3+	55.00	259.80	21+	81.00	351.80	23+	31.00	266.80	11+
Hanford fine sand	1.20	35.00	3+	4.20	75.00	5+	— 1.80	167.00	.....	10.20	82.00	12+
Tejunga sand	3.80	15.40	24+	2.80	55.40	5+	— .20	147.40	.....	— .20	62.40	.....
Yolo loam	— 2.00	123.20	.....	13.60	163.20	8+	42.00	255.20	16+	8.30	170.20	4+
Dublin clay loam	7.80	105.00	7+	11.80	145.00	8+	19.80	237.00	8+	75.80	152.00	49+
Dublin clay	9.95	131.60	7+	64.95	171.60	37+	47.95	263.60	18+	31.95	178.60	17+
Hanford sand	4.10	25.20	16+	3.80	65.20	5+	.....	157.20	.....	.20	72.20	Trace
Zelzah clay loam	9.00	109.20	8+	21.00	149.20	14+	3.00	241.20	1+	53.00	156.20	33+
Altamont loam	7.67	74.20	10+	14.67	114.20	12+	17.70	206.20	8+	23.67	121.20	19+
Diablo clay adobe	7.95	100.80	7+	16.15	140.80	11+	35.95	232.80	15+	19.95	147.80	13+
Altamont clay loam	.50	93.80	Trace	13.00	133.80	9+	17.00	225.80	7+	13.80	140.80	9+

The Pasadena area contains a larger percentage of soils having more than 0.1 per cent of nitrogen than does the Bay area. In the latter, for example, 26 per cent of the soils contained more than 0.1 per cent of nitrogen, while in the former more than 40 per cent of the soils belong in that class. Nevertheless it must still be observed that even the

Pasadena area falls short, approximately by 21 per cent, of having as large a number of soils with more than 0.1 per cent of nitrogen as the foreign-soil group. While, therefore, the soils of the Pasadena area approach more closely in nitrogen content those of the foreign group than do the soils of the Bay area, they are still distinctly inferior to the foreign soils. The effect of aridity of climate therefore makes itself plainly manifest in the Pasadena as it does in the Bay area series. It must be further remarked, however, that again owing to climatic conditions, a larger number of soils with less than 0.05 per cent of nitrogen are found in the Pasadena area than in the Bay area, and more of that class than among the foreign soils. The percentage of such is greater than 21 in the Pasadena area, as against 16 for the Bay area, and 14 for the foreign soils. Two soils out of the thirty-three in the Pasadena area contain less than 0.02 per cent of nitrogen, and two others contain less than 0.03 per cent of nitrogen.

On the basis of the absolute values for the nitrate from the soil nitrogen (Table IV, Group I) it would seem that the Pasadena area soils, though manifestly superior in nitrifying power as a class to the Bay area soils, are still far from being equal in that direction to the foreign soils. Thus, for example, 85 per cent of the soils in the Pasadena area produced less than 10 mgm. of nitrate nitrogen in 100 gm. of soil under the circumstances described, while the foreign soils numbered only 27 per cent of such soils among them. It is therefore very clear that so far as absolute quantities of nitrate produced are concerned even the fertile Pasadena area soils are inferior transformers when compared with foreign soils. On the other hand, when compared with the Bay area soils on the basis of a 5-mgm. production of nitrate, the Pasadena soils are clearly superior. Thus, among the latter there are 20 soils of the class last mentioned, whereas among the Bay area there is but 1 such soil.

On the relative basis, or that the criterion of which is percentage of soil nitrogen transformed into nitrate, the Pasadena area soils make even a better showing than on the absolute basis when compared with the Bay area soils. Likewise, they are less inferior when so judged in comparison with the foreign soils for obvious reasons concerned with the total nitrogen content. Thus 4 soils out of 33 (12 per cent) in the Pasadena area transforms more than 20 per cent of their total nitrogen content into nitrate as against 11 out of 44 soils of that class, or 25 per cent, in the case of the foreign soils. The highest individual record for percentage nitrogen transformation is attained equally by the Zelzah light loam and the Holland loam, which transform more than 26 per cent of the total nitrogen present into nitrate. This, while very high, is below the record attained by several of the foreign soils. All of these considerations, moreover, must be viewed in conjunction with the fact that even the group of foreign soils contains a few arid or semi-arid soils similar to the California soils which are being studied here.

Some very interesting data are available in Table IV, Group II, which gives the results with sulphate of ammonia. Thus, only about 21 per cent of the soils in the Pasadena area produced less nitrate from the sulphate of ammonia nitrogen plus the soil nitrogen than from the latter alone. It is interesting to note that the percentage of such soils is so nearly the same in the two arid-soil series thus far considered, even though the latter are in other respects very different. To emphasize again the wide difference existing in respect to the sulphate-of-ammonia nitrogen between the humid (as illustrated by the foreign) and the arid soils, one need but recall that 88 per cent of the humid soils failed to respond to sulphate of ammonia, whereas only 20 per cent of the arid soils behaved in that manner.

As other points of interest in Table IV, Group II, may be mentioned the following: (1) Only four soils in the whole series transform less than 5 per cent of the total nitrogen present into nitrates. (2) Six soils of the series transform more than 20 per cent of the total nitrogen present into nitrates. (3) In one soil, the Dublin clay, over 37 per cent of the total nitrogen present is nitrified, and another soil, the Hanford fine sandy loam, approaches closely to that record. (4) Both soils, the Zelzah light loam and the Holland loam, which have the highest record on the relative basis in Table IV, Group I (soil nitrogen alone), lose in nitrifying power very markedly when sulphate of ammonia is added to them, while the Dublin clay and the Hanford fine sandy loam, which do only moderately well with soil nitrogen alone, make the highest records, as above indicated, with soil nitrogen plus sulphate-of-ammonia nitrogen. (5) It should be noted that in the sulphate-of-ammonia series no soil loses its original nitrate content without replacing and adding to it by nitrification. A loss of the soil's original nitrate content does, however, occur in the case of two soils, the Chino loam and the Yolo loam, in the series with soil nitrogen alone. All of these points, moreover, are of great interest in comparison with the results for the Bay area soil series as obtained by the use of sulphate of ammonia. In general, of course, the superiority at nitrification of the soils in the Pasadena series to that of the Bay area series is more emphasized in Table IV, Group II, than heretofore.

In the experiments with dried blood in the case of the Pasadena area soils (see Table IV, Group III), results totally different in nature from those obtained with sulphate of ammonia are noted. Thus, 63 per cent of all the soils tested produce less nitrate under the circumstances above described when dried blood plus the soil nitrogen are available for nitrification than when only the soil nitrogen is present. This is 20 per cent in excess of the number of such soils in the Bay area, despite the fact that the latter area contains less soils than the Pasadena area with a percentage of nitrogen higher than 0.1. Again, however, as in the case of the Bay area, several of the soils produce in absolute quantities much

more nitrate than is produced by any of the same series when either soil nitrogen alone is present or when it is present with sulphate of ammonia. The number of such soils is about the same in the Pasadena as in the Bay area series, and in no one of them does the nitrogen content go quite as low as 0.1 per cent. They are, besides, soils of large internal surface throughout. Of the two other soils, which, in addition to the ones just mentioned, produce more nitrate in the blood series than in either of the foregoing, both are of large internal surface, and one contains very nearly 0.1 per cent of nitrogen, while the other contains nearly 0.075 per cent of nitrogen.

While on the absolute basis in the dried-blood series the Pasadena and Bay area soils are much alike, with the former in some respects superior and in other respects inferior to the latter, the difference is more marked on the relative basis. Thus, the records made for a percentage transformation of nitrogen into nitrate attain higher values in the Pasadena area than in the Bay area soils, and four soils of those above noted transform more than 20 per cent of the nitrogen present into nitrate, while three others pass the 15 per cent mark. It will be seen that there is only one soil in the Bay area even in the latter class in the dried-blood series. The foreign soils behave as a class of humid soils in a diametrically opposite manner from the Pasadena area soils with respect to dried blood. For the most emphatic proof of this, the reader can compare this paragraph with that discussing Table I, Group III.

Cottonseed meal gives in many respects results similar in the Pasadena area soils to those obtained with it in the Bay area soils, though in one or two respects the two are very different. Thus, for example, 26 per cent of the Bay area soils produce less nitrate from soil nitrogen plus cottonseed-meal nitrogen than from the former alone. The corresponding figure for the Pasadena area soils is 21 per cent. It is also interesting to observe that the last-named value is exactly or very nearly that of the analogous figure for the sulphate-of-ammonia series in the two soil areas above compared. Most striking of all are the very high absolute and almost necessarily high relative amounts of nitrates produced by many of the Pasadena area soils in the cottonseed-meal series. Thus, while there are among the foreign soils but two which transform more than 20 per cent of the total nitrogen in the soil and cottonseed meal into nitrate and none such in the Bay area soils, there are nine, or 28 per cent, of such soils in the Pasadena area group. Moreover, four of these nine transform, as indicated, more than 30 per cent of the nitrogen into nitrate, and one of these reaches the very high figure of a 57 per cent transformation. That cottonseed meal can be more readily and efficiently nitrified in the Pasadena soils as a class than it can in the foreign and the Bay area groups of soils is patent. Since, however, the Pasadena soils are under the most arid conditions of the three and

the Bay area soils are the intermediate group in that respect, it appears that cottonseed meal is better suited to arid than to humid soils. It may be well to note here that in the different groups of soils thus far studied the percentages of soils producing less nitrate from cottonseed meal plus soil nitrogen than from the latter alone are as follows: Foreign soils, 37 per cent; Bay area, 26 per cent; and Pasadena area, 21 per cent. The first figure is doubtless too low, because the foreign soils include several arid and semiarid soils which were not separated for purposes of this calculation.

RIVERSIDE AREA SOILS

Only 3 out of 52 soils of the Riverside area contain as much as 0.1 per cent of nitrogen, or more. That is equivalent to something over 5 per cent of the total number of soils and is strikingly low when contrasted with 66 per cent of such soils for the foreign soils, 40 per cent for the Pasadena area, and 26 per cent for the Bay area. What is even more striking is that including the three soils just mentioned there are but 8 soils (15 per cent) in the Riverside area which contain as much or more than 0.05 per cent of nitrogen. Over 36 per cent, or more than one-third of all the soils in this area, contain less than 0.03 per cent nitrogen. Two soils contain less than 0.01 per cent nitrogen and four others less than 0.015 per cent of nitrogen. Of the four groups of soils thus far studied, including the Riverside area, this last is clearly one in which the total nitrogen content is distinctly below that of all other groups. From the discussion already given this subject, such a circumstance should not be unexpected, since the general tendency is for more arid climates to produce soils with a lower nitrogen content than that of soils in a humid climate.

When the situation is reviewed with respect to nitrate formation from the soil-nitrogen supply in the Riverside areas (see Table V), some further interesting data become evident. In the first place it is plain that the absolute amounts of nitrate formed are very small and, while of a slightly greater magnitude than those of the Bay area, are still of about the same order. No soil in the whole area produces, under the conditions noted, as much as 10 mgm. of nitrate nitrogen, the largest amount produced being 7.40 mgm., produced by the Montezuma silty clay loam. Moreover, there are but 13 out of 53 soils, or about 24 per cent, which produce as much or more than 5 mgm. of nitrate nitrogen under the same conditions. There are, thus, more than three-fourths of the total number of soils tested in the Riverside area which produce less than 5 mgm. of nitrate nitrogen, and most of them form from 1 to 3 mgm. only. Here again, as in the case of the Bay area soils and the others, it appears impossible to find evidence for establishing a definite relation between

total nitrogen present and nitrate produced. Thus, for example, in the Riverside area, as in the Bay area, the largest amount of nitrate formed from the soil nitrogen is found in the soil with the highest total nitrogen content, but no nitrates are produced by the soil with the third highest total nitrogen content.

TABLE V.—*Nitrification in Riverside area soil types*

Name of type.	Soil nitrogen (Group I).			Soil nitrogen and sulphate of ammonia (Group II).			Soil nitrogen and dried blood (Group III).			Soil nitrogen and cottonseed meal (Group IV).		
	Nitrate produced	Total nitrogen present in soil.	Nitrogen nitrified.	Nitrate produced	Total nitrogen present in soil.	Nitrogen nitrified.	Nitrate produced	Total nitrogen present in soil.	Nitrogen nitrified.	Nitrate produced	Total nitrogen present in soil.	Nitrogen nitrified.
	Mgm.	*Mgm.*	*P. ct.*	*Mgm.*	*Mgm.*	*P. ct.*	*Mgm.*	*Mgm.*	*P. ct.*	*Mgm.*	*Mgm.*	*P. ct.*
San Joaquin sandy loam	1.80	32.20	5+	1.50	72.20	2+	−1.00	164.20	.....	10.00	79.20	12+
Placentia loam	5.80	42.00	13+	15.30	82.00	18+	47.90	174.00	27+	21.90	89.00	24+
Zelzah sandy loam	4.00	26.60	15+	40.00	66.60	60+	.....	158.60	.....	32.00	73.60	43+
Tejunga fine sandy loam	5.20	47.60	10+	47.20	87.60	53+	95.20	179.60	53+	50.40	94.60	53+
Sierra loam	.60	15.40	3+	1.50	55.40	2+	.....	147.40	.....	3.00	62.40	4+
Placentia sandy loam	5.18	22.40	23+	6.07	62.40	9+	−.12	154.40	.....	11.88	69.40	17+
Tejunga fine sand	3.92	28.00	7+	63.92	68.00	9+	−.08	160.00	.....	14.92	75.00	19+
Zelzah loam	3.74	32.20	11+	9.74	72.20	13+	8.54	164.20	5+	15.74	79.20	19+
Zelzah clay loam	3.40	53.20	6+	13.90	93.20	14+	32.10	185.20	17+	15.90	100.20	15+
Holland sandy loam	2.80	29.40	9+	5.00	69.40	7+	.....	161.40	.....	12.80	76.40	16+
Hanford loam	2.80	28.00	10+	6.40	68.00	9+	6.00	160.00	3+	20.00	75.00	26+
Hanford clay loam	6.80	74.20	9+	23.40	114.20	20+	63.60	206.20	30+	38.40	121.20	31+
Sierra loam	1.90	32.20	5+	4.80	72.20	6+	23.80	164.20	14+	11.80	79.20	14+
Hanford sandy loam	5.96	28.00	21+	9.36	68.00	13+	−.04	160.00	.....	6.96	75.00	9+
Hanford fine sandy loam	5.35	33.60	15+	15.75	73.60	21+	5.75	165.60	3+	31.75	80.60	39+
Hanford gravelly sandy loam	1.40	36.40	3+	17.40	76.40	22+	−.60	168.40	.....	27.40	83.40	32+
Hanford stony gravelly sandy loam	3.80	44.80	8+	12.60	84.80	14+	.....	176.80	.....	17.50	91.80	19+
Hanford loam	5.60	49.00	11+	27.60	89.00	31+	33.60	181.00	18+	63.60	96.00	66+
Hanford stony sand	2.35	5.60	41+	1.15	45.60	2+	−.05	137.60	.....	.95	52.60	1+
Hanford sandy loam	4.05	43.40	9+	8.85	83.40	10+	38.85	175.40	22+	27.85	90.40	30+
Tejunga sand	3.00	14.00	21+	1.90	54.00	3+	.....	146.00	.....	3.00	61.00	4+
Tejunga gravelly sand	1.52	11.12	13+	19.93	51.12	38+	−.08	143.12	.....	4.22	58.12	7+
Hanford sand	5.60	21.00	26+	.80	61.00	1+	.60	153.00	Tr.	2.80	68.00	4+
Hanford gravelly sand	4.10	37.80	10+	4.10	77.80	5+	−1.50	169.80	.....	15.20	84.00	18+
San Joaquin loam	2.00	35.00	5+	14.00	75.00	18+	1.60	167.00	Tr.	25.40	82.00	30+
Tejunga sandy loam	3.20	33.60	9+	7.20	73.60	9+	8.40	165.60	5+	15.20	80.60	18+
Hanford fine sandy loam	4.00	28.00	14+	12.00	68.00	17+	.90	160.00	Tr.	16.00	75.00	21+
Hanford gravelly loam	4.92	47.60	10+	7.92	87.60	9+	1.42	179.60	Tr.	13.92	94.60	14+
Placentia gravelly loam	5.00	36.40	13+	2.00	76.40	2+	.90	168.40	Tr.	.40	83.40	Tr.
Hanford stony loam	.....	110.60	.....	15.70	150.60	10+	47.40	242 60	19+	17.40	157.60	11+
Hanford coarse sandy loam	3.10	37.80	8+	4.00	77.80	5+	2.40	169.80	1+	24.00	84.80	28+
Montezuma loam	6.20	161.00	3+	44.00	201.00	21+	49.60	293.00	16+	32.50	208.00	15+
Montezuma clay adobe	4.45	33.60	13+	5.95	73.60	8+	2.15	168.60	1+	3.35	80.60	4+
Hanford coarse sand	1.35	15.40	8+	.45	55.40	Tr.	−.05	286.00	.....	.45	62.40	Tr.
Pedley fine sandy loam	2.40	32.20	7+	3.30	72.20	4+	.80	164.20	Tr.	6.50	79.20	8+
Montezuma silty clay loam	7.40	182.00	4+	46.40	222.00	20+	70.40	314.00	22+	38.40	229.00	16+
Hanford coarse sandy loam	1.85	21.00	8+	1.95	61.00	3+	−.15	153.00	.....	3.85	68.00	5+
Olympic loam	3.60	40.60	8+	6.00	80.60	7+	4.40	172.60	2+	4.00	87.60	4+
Oakley sand	3.00	26.60	11+	6.40	66.60	9+	.....	158.60	.....	1.30	73.60	1+
Tejunga stony sand	.....	.....	.....	3.10	.....	.....	1.40	.....	.....	35.00	.....	.....
Kimball fine sandy loam	2.40	9.80	24+	8.00	49.80	16+	5.00	141.80	3+	18.00	56.80	31+
Aiken loam	6.00	50.40	11+	7.00	90.40	7+	20.00	182.40	10+	14.20	97.40	14+
Mendocino loam	3.00	21.00	14+	5.20	61.00	8+	16.00	153.00	10+	7.50	68.00	11+
Holland fine sandy loam	4.00	39.20	10+	10.00	79.20	12+	.50	171.20	Tr.	15.60	86.20	18+
Corona gravelly sandy loam	5.10	54.60	9+	13.80	94.60	14+	58.40	186.60	31+	28.40	101.60	27+
Corona clay loam	3.80	50.40	7+	5.00	90.40	5+	20.00	182.40	10+	14.00	97.40	14+
Yolo gravelly loam	4.00	47.60	8+	7.00	87.60	7+	32.00	179.60	17+	15.80	94.60	16+
Rincon loam	2.80	39.20	7+	3.20	79.20	4+	4.00	171.20	2+	3.00	86.20	3+
Antioch silty clay loam	3.80	35.00	10+	7.80	75.00	10+	26.30	167.00	15+	14.80	82.00	18+
Hanford silty clay loam	1.50	28.00	5+	5.00	68.00	7+	2.10	160.00	1+	2.80	75.00	3+
Placentia clay	2.00	42.00	4+	8.00	82.00	9+	12.00	174.00	6+	14.60	89.00	16+
Sierra sandy loam	4.80	25.20	19+	5.20	65.20	7+	.70	157.20	.....	13.20	72.20	18+
Holland loam	2.40	28.00	8+	3.00	68.00	4+	.....	160.00	.....	9.20	75.00	12+

Owing to the very low total nitrogen content of the Riverside area soils, the relative figures for nitrate transformation given in Table V, Group I, are, if wholly expected, exceedingly large in many instances. Thus, there are six soils, or 11 per cent of the whole number studied, which nitrify more than 20 per cent of the total nitrogen present and 24, or 46 per cent of all the soils, which transform more than 10 per cent of the total amount of nitrogen present into nitrate. On the relative basis, therefore, the Riverside area soils are far superior in nitrifying power to the Bay area soils, but not so in relation to the Pasadena soils; and when compared with the foreign, consisting very largely of humid soils, the Riverside soils are much inferior even on the relative basis.

The results obtained with the soils of the Riverside area employing sulphate of ammonia are perhaps the most striking of all thus far studied. Thus, on the absolute basis there are but 6 soils, or a little over 11 per cent of the whole number tested, which yielded less nitrate (see Table V, Group II) with the combined nitrogen of the soil and of the sulphate of ammonia present than from the former alone. It will be recalled that the corresponding figures for the foregoing series were as follows: Foreign soils, 87 per cent; Bay area soils, 16 per cent; and Pasadena area soils, 21 per cent. In other words, it would appear that the more distinctly arid a soil's character is the more likelihood there is of its being favorably affected by sulphate of ammonia, or to put it otherwise, to have its nitrifying flora stimulated to greater activity. It will be noted that the 6 soils of the Riverside area which were unfavorably affected as to nitrifying power by the sulphate of ammonia are all either sandy, coarse sandy, or gravelly soils. The actual amounts of nitrate produced in many of the soils of this series under the influence of sulphate of ammonia are very large—for example, there are 5 soils which produce more than 40 mgm. of nitrate nitrogen under the conditions of the experiment and 2 other soils which produce more than 20 mgm. of nitrate nitrogen. There are 18 soils in the area, or more than 33 per cent of the whole number studied, which produce more than 10 mgm. of nitrate nitrogen, and 1 soil which produced as much as 63.92 mgm. of nitrate nitrogen. It will be remembered, however, that even this record is surpassed by one of the Pasadena area soils with sulphate of ammonia and that much larger absolute productions of nitrate nitrogen are accomplished with other forms of nitrogen in a number of instances by the foreign soils.

Partly as a result of the high absolute quantities of nitrate produced by soils in the Riverside area and partly owing to the relatively small quantity of total nitrogen present, the percentages given in the last column of Table V, Group II, make a very good showing. Thus, there are 10 soils, or about 18 per cent of the number tested, which transform in every case more than 20 per cent of the total nitrogen present into

nitrate, and 23 soils, or about 43 per cent of the whole number in this area, which nitrify 10 per cent or more of the nitrogen present. When compared with 66 per cent of the soils with such a record among the foreign group with the soil nitrogen alone, it still seems clear that the arid soils are inferior as nitrifying media. Nevertheless one remarkable figure must not be lost sight of among the relative data of Table V, Group II—that is, the 94 per cent transformation of the total nitrogen present into nitrate by the Tejunga fine sand. This means an almost complete nitrification of both soil nitrogen and sulphate of ammonia nitrogen added in one month under the conditions noted.

In the case of dried-blood nitrogen, the Riverside area soils again behave typically. Fifty per cent of all the soils produce in the absolute, as reference to Table V, Groups III and IV, will show, less nitrate from dried blood plus soil nitrogen than from the latter alone. In the Riverside area, however, even more markedly than in the two preceding California areas, there is a considerable number of soils producing large quantities of nitrates from soil plus dried-blood nitrogen. Two points of difference are noted between the Riverside area soils on the one hand and the two groups just mentioned on the other. There are more soils of the class just described in the Riverside area soils than in the Bay area or Pasadena area soils, and very few of them contain nearly as much as 0.1 per cent of total nitrogen. Besides, the absolute quantities of nitrate produced are in four or five cases exceptionally high. It must be noted, however, that in only one case does the nitrification of dried blood exceed that of the soil nitrogen when the soil is stony, sandy, or gravelly and contains little loam or clay. Even that one exception shows but feeble powers of nitrification. In the other cases the soils vary from fine sandy loams with large internal surface to clay loams and clays with larger internal surface. Likewise, it will be noted that in the cases of the 3 soils with more than 0.1 per cent total nitrogen every one made a very good showing in the nitrification of dried-blood nitrogen. It will further be observed that of the 12 soils producing more than 25 mgm. of nitrate nitrogen in this series only one contained less than 0.04 per cent of total nitrogen.

On the relative basis it follows that the Riverside area soils must surpass the Bay area soils in efficiency in the dried-blood series and that they must equal the Pasadena area soils, but they do not do either with respect to the foreign soils. For example, five soils in the group now under consideration transform more than 20 per cent of the blood plus soil nitrogen into nitrate, and six more transform more than 15 per cent of the total nitrogen present in that manner. This is a record only slightly behind that of the Pasadena area soils, but as far behind that of the foreign soils as it is ahead of the Bay area soils. So far as maximum transformation is concerned, however, the Tejunga fine sandy loam in the Riverside area surpasses any soil in the Pasadena area by nitrifying

53 per cent of the total nitrogen of soil and dried blood under the conditions of the experiment. In comparison with the foreign soils as regards dried blood, the Riverside soils must be regarded in the same light as the Pasadena soils, which will be shown more fully later.

Much the same situation as exists in the cottonseed-meal series of the Pasadena area soils is to be found in the Riverside area soils (see Table V, Group IV). There are two principal differences between them. One is that there are only 11 per cent of the Riverside soils, as against 21 per cent of the Pasadena soils, which produce less nitrate in the absolute from cottonseed meal plus soil nitrogen than from the latter alone. The other is that, on the whole, more vigorous nitrification of cottonseed-meal nitrogen occurs in the Riverside than in the Pasadena soils. The latter superiority is based mainly on the fact that more than 17 per cent of all the soils in the Riverside area transform more than 30 per cent of the total nitrogen present into nitrate, whereas the corresponding figure for the Pasadena area is 13 per cent. It may be added also in connection with the preceding that the highest percentage transformation of cottonseed meal plus soil nitrogen found anywhere among the California soil series studied occurs in the Riverside area soil known as the Hanford loam, the record being 66 per cent. On the whole, therefore, the Riverside area soils are the most efficient of any in the nitrification of cottonseed meal plus soil nitrogen. This result is further strengthened by the fact that nearly 70 per cent of all the soils present produce 10 mgm. or more of nitrate nitrogen in this series, as shown in Table V, Group IV. It is clear also that in this group there is further evidence of the definite relationship of degree of aridity in climate to its effect on the nitrifying power of soils for a given form of nitrogen.

THE UKIAH AREA SOILS

Table VI gives the results obtained in our experiments with the Ukiah area soils which were carried out in a manner similar to those described for the other series. Of the Ukiah series, 10 soils, or over 35 per cent of the whole number, contain more than 0.1 per cent of nitrogen. This is 9 per cent in excess of the corresponding figure for the Bay area, which ranks highest in the areas studied. Of the 10 soils just mentioned, 6 contain more than 0.14 per cent of nitrogen, a marked contrast to the corresponding number for the Bay area, which is 1. Nevertheless, while among all the California soil series here studied the Ukiah area stands easily first with respect to the number of soils containing more than 0.1 per cent of total nitrogen, it still falls short more than 26 per cent of equaling the record in the same regard attained by the foreign soils. That, as compared with the other California series, the Ukiah area soils receive the place to which theoretically they should be entitled on the basis of total nitrogen content is also indicated in Table VI. Thus,

it is clear that the Ukiah area should stand first among the California series here studied. This is so because it receives more rain in a longer rainy season and is not subjected to the extremely high tenperatures and other conditions favorable to loss of nitrogen which exist in the more arid areas. There are only three soils in the Ukiah area, or about 10 per cent of the whole number, which contain less than 0.05 per cent of total nitrogen. Two of these approach that point rather closely and the other contains nearly 0.03 per cent of nitrogen and is the only soil which is seriously deficient in nitrogen in the whole area. The 10 per cent value just given, when compared with corresponding values for other California areas, again supports the relationship drawn above between climate and soil nitrogen content and which for the first time is being properly emphasized.

TABLE VI.—*Nitrification in Ukiah area soil types*

Name of type.	Soil nitrogen (Group I).			Soil nitrogen and sulphate of ammonia (Group II).			Soil nitrogen and dried blood (Group III).			Soil nitrogen and cottonseed meal (Group IV).		
	Nitrate produced	Total nitrate present in soil.	Nitrogen nitrified.	Nitrate produced	Total nitrogen present in soil.	Nitrogen nitrified.	Nitrate produced	Total nitrogen present in soil.	Nitrogen nitrified.	Nitrate produced	Total nitrate present in soil.	Nitrogen nitrified.
	Mgm.	*Mgm.*	*P. ct.*	*Mgm.*	*Mgm.*	*P. ct.*	*Mgm.*	*Mgm.*	*P. ct.*	*Mgm.*	*Mgm.*	*P. ct.*
Ukiah gravelly loam	0.10	156.80	Tr.	—0.40	196.80	.....	0.60	288.80	Tr.	0.50	203.80	Tr.
Vichy fine sandy loam	.02	29.40	Tr.	— .28	69.40	.....	.12	161.40	Tr.	.52	76.40	Tr.
Russian silt loam	1.42	91.00	1+	2.42	131.00	1+	1.82	223.00	Tr.	1.82	138.00	1+
Pinole gravelly loam	2.94	77.00	3+	3.64	117.00	3+	— .06	209.00	.....	3.54	124.00	2+
Finn gravelly loam	.30	70.00	Tr.	.44	110.60	Tr.	1.90	202.00	Tr.	.20	117.00	Tr.
Orr loam	4.79	75.60	6+	15.79	115.60	13+	6.29	207.60	3+	2.19	122.60	1+
Large gravelly loam	2.47	203.00	1+	4.37	243.00	1+	18.97	335.00	5+	8.77	250.00	3+
Russian silty clay loam	5.50	147.00	3+	7.50	187.00	4+	28.50	279.00	10+	12.00	194.00	6+
Russian silt loam	2.08	140.00	1+	5.58	180.00	3+	29.58	272.00	10+	8.58	187.00	4+
Largo silty clay	3.63	152.60	2+	6.93	192.60	3+	20.93	284.60	7+	9.53	199.60	4+
Largo loam	6.10	163.80	3+	12.00	203.80	5+	23.60	295.80	7+	13.60	210.80	6+
Sanel loam	.48	58.80	Tr.	.28	98.80	Tr.	— .02	190.80	.....	.07	105.80	.....
Guidiville sandy loam	3.59	42.00	8+	8.39	82.00	10+	— .41	174.00	.....	14.79	89.00	16+
Tehama loam	.03	49.00	Tr.	— .12	89.00	.....	.98	181.00	Tr.	.36	96.00	Tr.
Russian fine sandy loam	.85	53.20	1+	8.35	93.20	8+	8.35	185.20	4+	8.35	100.20	8+
Diablo clay	2.39	63.00	3+	13.89	103.00	13+	11.89	195.00	6+	21.49	110.00	14+
Finn loam	1.42	61.60	2+	1.32	101.60	1+	— .08	193.60	.....	.72	108.60	Tr.
Dublin clay	3.52	147.00	2+	5.82	187.00	3+	23.92	279.00	8+	10.32	194.00	5+
Russian loam	6.45	120.40	5+	10.95	160.40	6+	— .05	252.40	.....	5.15	167.40	3+
Largo silt loam	7.00	135.80	5+	10.80	175.80	6+	4.30	267.80	1+	4.00	182.80	2+
Mendocino loam	2.69	61.60	4+	3.99	101.60	3+	— .01	193.60	.....	.01	108.60	.....
Tehama gravelly loam	1.00	67.20	1+	1.20	107.20	1+	6.60	199.20	3+	.32	114.20	Tr.
Finn gravelly clay loam	1.24	75.60	1+	.28	115.60	Tr.	1.64	207.60	Tr.	1.04	122.60	Tr.
Pinole loam	3.00	54.60	5+	2.40	94.60	2+	.....	186.60	.....	.68	101.60	Tr.
Diablo loam	3.30	85.40	3+	2.90	125.40	2+	.02	217.40	.....	2.58	132.40	1+
Orr clay loam	5.10	112.00	4+	13.70	152.00	9+	39.70	244.00	16+	19.70	159.00	12+
Pinole sandy loam	2.72	85.40	3+	5.62	125.40	4+	— .08	217.40	.....	9.02	132.40	6+
Mendocino gravelly loam	5.46	95.20	5+	15.66	135.20	1+	26.66	227.20	11+	11.86	142.20	8+

With reference to the absolute amounts of nitrate produced from soil nitrogen, it will be seen that the Ukiah soils are distinctly superior to the Bay area soils. Thus, 6 soils in the Ukiah area produce more than 5 mgm. of nitrate nitrogen, as against only 1 in even a larger total number of soils in the Bay series. Moreover, 1 of the 6 soils under discussion reaches a nitrate production equivalent to 7 mgm. Two soils in the

Ukiah series produce almost no nitrates, but no soil loses nitrates during the period of incubation. The average nitrate production is 2.84 mgm. in the Ukiah series and 2.09 mgm. in the Bay area series. The data for the percentage transformation of nitrogen into nitrates in the Ukiah series are also correspondingly larger than those of the Bay area, as is the case with the absolute data. Thus, an 8 per cent maximum transformation is attained in the Ukiah series, as against one of 6 per cent in the Bay area series, and 5 soils besides transform more than 5 per cent of the nitrogen present into nitrate, as against 4 such in the Bay area.

Table VI, Group II, helps to emphasize again the several points made in the foregoing discussion with respect to the behavior of sulphate of ammonia in the Bay area soils. Of the total number of soils in the Ukiah area, 8, or about 28 per cent, produce less nitrate from sulphate of ammonia plus soil nitrogen than from the latter alone. The corresponding figure for the Bay area is 20 per cent. From these facts, it appears that parallelism between degree of aridity of climate and the nitrifying power of soils for different forms of nitrogen is more firmly supported than ever. Thus, the Ukiah area soils as a class having more nitrogen and greater internal surface act more nearly like the humid soils than any of the other California series here studied, even though they are still far removed from the humid soils in that direction. Just as sulphate of ammonia stimulates nitrification in the Bay area soils, so it it does in commensurate degree in the Ukiah area soils. As the average absolute nitrate production is higher from soil nitrogen alone in the Ukiah as against the Bay area group of soils so it is with respect to the sulphate-of-ammonia series of the two soil groups. The maximum transformation of nitrogen into nitrate is also a little higher in the sulphate-of-ammonia series with the Ukiah soils, it being 13 per cent in the case of two soils, as against 11 per cent, which was the corresponding figure in the Bay area soils. On the other hand, there are about equal numbers of soils in the two areas (4 or 5) which transform more than 9 per cent of the total nitrogen present in this series into nitrate. The other soils are all considerably more feeble in nitrification. The average nitrogen transformation on the relative basis is only slightly greater in the Ukiah than in the Bay area soils with sulphate of ammonia as the nitrifiable material.

Only 35 per cent of the soils in the Ukiah area produce less nitrate from combined soil and dried-blood nitrogen than from the former alone (see Table VI, Group III). This is in sharp contrast to the Bay area soils, for which the corresponding figure is 50 per cent, and with the Pasadena area, for which the corresponding value is 63 per cent. All of these, moreover, are in sharp contrast to the corresponding value for the foreign soils, which is 20 per cent. It will be noted next that very large nitrate productions are accomplished in the dried-blood series. Such quantities are in all cases much in excess of those produced by the same

soils in the sulphate-of-ammonia or the soil-nitrogen series. Thus, 8 soils in a total of 28 produce more than 20 mgm. of nitrate nitrogen under the conditions of the experiment. Allowing for one or two exceptional soils, the break between the high nitrate-producing soils and the rest in the blood series is very abrupt. As against the 8 soils just referred to in the Ukiah series, there are but 3 in the Bay area series of a corresponding class. In general, nitrification of dried-blood nitrogen proceeds very much better in the Ukiah series than in the Bay area soils. In fact, the discrepancies in nitrifying powers between the two soil groups are better exemplified with dried blood than with any other form of nitrogen. With one exception, all the soils in the dried-blood series which produce more than 20 mgm. of nitrates contain considerably more than 0.1 per cent of nitrogen, and are all possessed of large internal surface. The one exception mentioned is Mendocino gravelly loam, which contains very nearly 0.1 per cent of nitrogen and has a large internal surface besides. Owing to the higher nitrogen content in many of the soils of the Ukiah area than in corresponding soils of the Bay area, the relative figures for the two areas using dried-blood nitrogen as the nitrifiable material do not differ as much as the absolute figures. Nevertheless the relative values are again distinctly in favor of the Ukiah area soils.

A study of Table VI, Group IV, brings us to a consideration of cottonseed-meal nitrogen in its relations to the soils of the Ukiah area. As a result of such consideration we find that 40 per cent of the soils concerned produce less nitrate from cottonseed meal plus soil nitrogen than from the latter alone in every case. This is a behavior with respect to cottonseed-meal nitrogen very similar to that evinced by the foreign soils, the corresponding percentage for which was 37. The latter is obtained even with a few arid and semiarid or otherwise peculiar soils included, as previously pointed out. On the other hand, the corresponding figure for the Bay area soils is 26 per cent, and it is much lower for the other areas. In other words, it would seem that the Ukiah soils not only approach very closely in their behavior to cottonseed meal that of humid soils, but also that the curve in that respect shows a gradual decline with an increase in the aridity of the climate concerned. The maximum absolute production of nitrate is 29.49 mgm. as seen in Table VI, Group IV, accomplished by the Diablo clay. This record is surpassed in two cases in the Bay area series, and nearly equaled in two others.

It may be well to summarize briefly from one or two points of view the results which were obtained in the experiments with the California soils. In a similar manner, therefore, to that employed in Table II for the foreign soils, Table VII gives for California the percentage of soils in each area which transformed more than 10 per cent of the total nitrogen in a given culture into nitrate, giving every form of nitrogen separately.

TABLE VII.—*Percentage of California soils transforming more than 10 per cent of nitrogen present in culture into nitrate*

Form of nitrogen.	Soil area.			
	Riverside.	Pasadena.	Bay.	Ukiah.
Soil nitrogen alone	47	34	0	0
Soil nitrogen plus ammonium sulphate	47	47	6	10
Soil nitrogen plus dried blood	30	28	10	14
Soil nitrogen plus cottonseed meal	70	50	20	10

It appears, therefore, that in the Riverside, Pasadena, and Bay areas the cottonseed-meal nitrogen gives the best results in the largest number of soils. In the Ukiah area cottonseed meal takes second place and divides honors with sulphate of ammonia. Sulphate of ammonia takes second place in the Riverside, Pasadena, and Ukiah area soils, but third in the Bay area soils. The soil nitrogen does, however, contend with it for second place in the Riverside soils. Dried blood is last in both the Pasadena and Riverside soils, but first in the Ukiah soils and second in the Bay area soils. Soil nitrogen is either second or third in the Riverside soils, is third in the Pasadena soils, and last in the other two areas.

Table VIII gives relative values on another basis than that employed in Table VII. This latter criterion consists in computing the percentages of soils which produce with every form of nitrogen more than 15 mgm. of nitrate in the foreign soils and is based on the amounts of total nitrogen present in corresponding quantities in the other soil areas. Thus, the figure is 11 instead of 15 for the Ukiah area, 10 for the Bay and Pasadena areas, and 5 for the Riverside area.

TABLE VIII.—*Percentages of soils producing more than 15 mgm. of nitrate in foreign soils with every form of nitrogen*

Form of nitrogen.	Soil area.				
	Riverside.	Pasadena.	Bay.	Ukiah.	Foreign.
Soil nitrogen alone	24	18	0	0	52
Soil nitrogen plus ammonium sulphate	71	71	16	25	25
Soil nitrogen plus dried blood	41	42	30	31	81
Soil nitrogen plus cottonseed meal	75	80	23	21	63

Similar relations appear to hold in this manner of computation as in that previously employed. Some minor differences, however, are apparent. Ammonium sulphate very definitely takes second place in all the California soils except the Bay area. Cottonseed meal is still first in the Riverside and the Pasadena soils, but blood is first in the Ukiah and Bay area soils. The soil nitrogen is fourth in all the California soil areas.

In addition to these observations, it may be remarked that in the last column, in the foreign soils, dried blood stands first, as it does in the Bay and Ukiah areas, and soil nitrogen stands third. Sulphate of ammonia in the foreign soils again takes last place instead of a close second, as in the Riverside and Pasadena areas, and cottonseed meal stands second instead of first.

COMPARISON OF FOREIGN AND CALIFORNIA SOILS

It has been shown that 52 per cent of the foreign soils, which include several arid or semiarid soils, produce more than 15 mgm. of nitrate nitrogen out of the total soil nitrogen present. Neither the Bay nor the Ukiah areas includes any soil of such nitrifying activity. However, it was not expected that they would produce the same number of milligrams of nitrate nitrogen, but merely an amount having an approximately similar ratio to the total nitrogen as that in the foreign soils. While the Riverside and the Pasadena areas, with 24 per cent and 18 per cent, respectively, of soils with an equivalent nitrifying power to that of the foreign soils mentioned, are much more active than those of the other two arid-soil areas just referred to, their records are still far behind those of the foreign soils. These comparative data were arranged as noted on the basis of equivalent quantities of total nitrogen, a basis employed because of the claim which has been made that the quantity of nitrogen rendered available in a soil is always a certain constant proportion of the total nitrogen present in soils. If the latter theory is tenable, arid soils are certainly very much more feeble in the nitrification of soil nitrogen than humid soils. But if the theory above mentioned is incorrect, the data are all the more emphatic as to the considerable disparity (in favor of the humid soils) between the nitrifying power for soil nitrogen of soils of the arid and humid regions.

On the assumption that the stimulating or depressing effect of a certain nitrogenous material on the soil's nitrifying power under laboratory conditions is a justifiable criterion, the percentage of soils in every group which produced less or more nitrate from soil plus fertilizer nitrogen than from soil nitrogen alone have been noted. Table IX has been arranged on the basis of all those calculations.

TABLE IX.—*Percentage of soils in every area which produced less nitrate with fertilizer plus soil nitrogen than from soil nitrogen alone*

Soil.	Source of nitrogen.		
	Sulphate of ammonia and soil.	Dried blood and soil.	Cottonseed meal and soil.
Foreign	88	20	46
Ukiah	28	35	40
Bay	16	43	26
Pasadena	21	63	21
Riverside	11	50	11

It seems clear from Table IX that sulphate of ammonia induces under laboratory conditions a larger yield of nitrate in the large majority of the soils of arid regions than could be produced by those soils with their own nitrogen. The range in the percentage of such soils is not large and varies between 89 per cent in the Riverside area and 72 per cent in the Ukiah area. On the other hand, it is clear also that in the large majority of soils in the foreign group ammonium sulphate has the opposite effect—that is, to depress the nitrifying power of a soil for its own nitrogen. The effects of sulphate of ammonia are thus almost exactly reversed in a comparison of the Riverside and the foreign soils, for example. In the comparison made by Sackett (10) of the nitrifying power of certain Colorado soils (using ammonium sulphate) with certain others from several other States, it will be noted that the foreign soils, with the exception of the California soils, acted as a class like those studied by the writers. Likewise, the Colorado soils are more like the arid soils which were described previously. In other words, the Colorado soils were superior in nitrifying power for sulphate-of-ammonia nitrogen to soils from other States, even not excepting the California soils. It must be remembered, however, that anything more than a general analogy or comparison between the writers' results and Sackett's is not permissible for the following reasons: Sackett used 100 mgm. of ammonium-sulphate nitrogen, while the writers used 40 mgm. in the soil cultures. He inoculated the cultures with a fresh soil suspension in the case of every soil studied, which was equivalent to 5 gm. of soil, while the writers merely employed the soil flora existing in the air-dried soil. Sackett used a six weeks' period of incubation, whereas the writers employed only a four weeks' period. In several other minor respects the conditions of Sackett's experiments were different, among which may be particularly mentioned the method of analysis employed for nitrate determinations.

In the case of dried blood, conditions seem to be almost the reverse of those with ammonium sulphate. The soils of the foreign group which produce less nitrate from dried blood than from their own nitrogen alone are decidedly in the minority, but the reverse is true of the arid soils of California as they become more and more humid in character. Dried blood therefore induces a loss in nitrate-producing power in from 35 to 63 per cent of the soils of the arid region. On the other hand, the same substance affects only 20 per cent of the total number of foreign soils in that manner. Hence, there is a stimulation to nitrification in some soils of the arid region induced by the presence of dried blood in them. The opposite is true with humid soils.

With respect to the stimulating or retarding action on the nitrifying powers of the soils studied, cottonseed meal acts almost exactly like ammonium sulphate in the Pasadena and Riverside areas. The difference between the two materials is, however, much greater in the other two arid-soil groups; but still in both cases the percentage of soils in which apparent losses in nitrifying power are induced is below that of the foreign

group. The latter is none the less almost half the magnitude of the corresponding figure for sulphate of ammonia with the foreign soils.

From all the foregoing considerations it appears evident that different forms of nitrogen exercise widely different effects on different groups of soils. Thus, for example, ammonium sulphate seems to enhance the nitrifying powers of arid soils and to depress those of humid soils. Dried blood seems, in general, to operate in a reverse manner, while cottonseed meal seems to act more like the ammonium sulphate. It will be remembered that conclusions similar to those reached for arid soils were drawn by Lipman and Burgess (7) with respect to another group of arid soils with which they worked. Moreover, Sackett, in the experiments cited, noted that the relationship of the Colorado soils to sulphate of ammonia and to dried blood was the reverse of the relation of the other soils which he studied to those substances. The position of the Colorado soil was similar to that occupied by the California soils with respect to the foreign soils. The apparently mystifying feature of the comparison lies, however, in the fact that the California soils tested were in Sackett's foreign group (10) and, therefore, in his hands gave different results from those obtained by us. This may perhaps be accounted for by the fact that the foreign soils are considered as a whole and are not separated from the California soils, which in reality give much better results than, for example, the eastern soils with sulphate of ammonia.

Table X has been arranged to bring together some of the figures above discussed with respect to the relative powers of soils to nitrify different forms of nitrogen. The first group shows the percentage of soils in each of the areas studied which transformed more than 10 per cent of the total nitrogen present into nitrate in the case of every form of nitrogen employed. The second group shows the percentage of soils in every area which produced a quantity of nitrate equivalent to 15 mgm. in the case of the foreign soils. The equivalent amount for soils other than those of the foreign group was determined by using the same ratio of nitrate to total nitrogen which is employed in the last-named groups of soils, the average total nitrogen content of all soils in a given area being used as a basis.

TABLE X.—*Transformation of nitrogen in various soil areas*

Form of nitrogen.	Percentage of soils which transformed 10 per cent or more of nitrogen into nitrate.					Percentage of soils producing 15 mgm. of nitrate nitrogen in the foreign area and an equivalent quantity based on nitrogen present in other areas.				
	Riverside area.	Pasadena area.	Bay area.	Ukiah area.	Foreign area.	Riverside area.	Pasadena area.	Bay area.	Ukiah area.	Foreign area.
Soil nitrogen only	47	34	0	0	68	24	18	0	0	52
Soil nitrogen and ammonium sulphate	47	47	6	10	23	71	71	16	25	25
Soil nitrogen and dried blood	30	28	10	14	47	41	42	30	31	81
Soil nitrogen and cottonseed meal	70	60	20	10	45	75	80	23	21	63

By the different arrangement of the data in Tables VIII to X a reversal of indications in minor ways has perhaps been brought about. But, in general, certain differences of a marked character in the nitrifying powers of humid and arid soils are obvious. Thus, by whatever method compared, the soil nitrogen of humid soils seems to become nitrified more readily than that of arid soils. Likewise, the opposite is true of sulphate-of-ammonia and cottonseed-meal nitrogen and their effects on the nitrification of soil nitrogen, and, as pointed out, the difference may amount to veritable inverse relationships. On the other hand, the opposite of the effects noted for the forms of nitrogen just discussed is true in general for dried-blood nitrogen. The most marked differences are, of course, evident between the foreign soils on the one hand and the Pasadena and Riverside soils on the other, because the latter are more distinctly arid in character than the Ukiah and Bay soils.

REVIEW OF RESULTS

Since the results obtained by the writers are very striking and offer the first direct evidence, so far as they are aware, of the differences between the nitrifying powers of humid and arid soils, it is essential that their findings be viewed critically. The first question which arises is that of the representative nature of the samples of soil employed with respect to the different climatic regions. The four soil areas chosen to represent arid soils in California may be taken as representative because they exemplify as nearly as possible interior valley and coast conditions in both southern and northern California. Since more than twice as much rainfall is normally received by the Bay and Ukiah areas as that received by the Riverside and Pasadena areas, these soils, all of which are none the less arid, should be considered as representative of average conditions in the State. If, however, it is desired to apply the term "arid" only to regions receiving less than 20 inches of rain a year, it is necessary to consider only the Riverside and Pasadena soils. So far as soil nitrogen and nitrification are concerned, the Riverside area will fairly represent a large, if not the largest, part of the San Joaquin Valley, a small part of the Sacramento Valley, the Coachella and Imperial Valleys, and nearly all of the Mojave and the Colorado Desert regions besides. The Pasadena and the Bay areas may be taken as nearly representative of the coast valley conditions from San Francisco to San Diego, the first being only in behavior more typical of the southern and the second of the northern valleys. The Ukiah area will partly represent a large portion of the northern half of Sacramento Valley and much of the coast region above San Francisco. In general, therefore, the four soil areas are fairly representative of California conditions, and in particular may serve, as above indicated, to represent the typically arid conditions of the State if only a certain area or areas be chosen.

In regard to the humid soils, the situation is by no means as satisfactory. As already indicated, the soil samples from the other States were sent by Experiment Station officers and may therefore, in many instances at least, have been chosen from exceptionally well-managed fields. On the other hand, it would not be fair so assume that the land of every State Experiment Station is the best to be had in the State. It is unlikely that the samples here studied would represent anything more than average conditions of the Eastern States. It must be added, of course, that when the soils of individual States of the humid group are considered, most of them may be found either very deficient or very excellent in respect to nitrifying power. Such of the Eastern States, however, as contain soils throughout of a low nitrifying power are decidedly in the minority. It is therefore gratifying to be able to point to the relatively low nitrifying power of the Sassafras loam from New Jersey, as given in Table I, and to show in Table XI the nitrifying powers, similarly determined, of a number of other New Jersey soil types for comparison. It was fortunate that these samples of soil were made available from an exhibit sent to the Panama Pacific Exposition by the New Jersey Experiment Station.

TABLE XI.—*Comparative nitrifying power of soil types from New Jersey*

Name of type.	Nitrate produced.	Total soil nitrogen present.	Soil nitrogen nitrified.
	Mgm.	*Mgm.*	*Per cent.*
Hoosic gravelly loam	4.30	149.80	2+
Norfolk sand	.10	15.40	.....
Colbington sandy loam	3.75	103.60	3+
Dover loam	5.30	121.80	4+
Dutchess loam	.30	81.20	.....
Sassafras loam	2.40	131.60	1+
Penn loam	4.10	99.40	4+
Light sandy loam	2.50	96.60	2+
Do	3.40	74.20	4+

Since the samples described in Table XI were kept in a dry condition in sacks for considerably over a year, it is possible that the soils may have lost to a relatively slight degree their powers to nitrify their own supply of nitrogen, and the figures obtained might be regarded as a little low on that account. This effect on the New Jersey soils of drying could not have been very great, as the evidence of the other soils would show. It is therefore seen that of nine types of New Jersey soils studied, not one has the power to change much more than 4 per cent of the total nitrogen present into nitrate, and the samples described in Table I transformed only a little more than 6 per cent of the soil nitrogen into nitrates. It is interesting to note that the last-named sample, while classified as a Sassafras loam, is very different in total nitrogen content

and in nitrifying power from the sample of the same name described in Table XI. This question of variation in any one type is now being investigated.

In general, therefore, it appears possible that some States may contain very few good nitrifying soils, others very few poor nitrifying soils, and still others contain a fair proportion of both. If this possibility is allowed, then the samples described in this paper as the foreign soils must approach closely the average conditions of humid soils, despite the criticisms which are above suggested.

The next question is that of the influence of the seasonal variation in the nitrifying activity of soils on the validity of nitrification data, and particularly of those above presented. As pointed out in the introductory part of this paper, there are now in the writers' possession many data on the monthly variation in the nitrifying powers of several different soil types. These data, which cover a period of 1½ years of monthly tests, indicate even more strikingly than former results in this regard the great variability to which such determinations are subject. Hence, the low nitrifying powers of the Bay and Ukiah area soils which were gathered from November to December, 1914, may be regarded as due to the depressing effect of the conditions of the late fall, particularly as regards a lack of moisture. It is also realized that the much greater relative activity of the soils of the Pasadena and Riverside areas may in part be accounted for by the collection of the samples in June and July, when conditions were more favorable to nitrification. The humid soils were collected in various parts of the year, but the bulk of them arrived at Berkeley between September 15, 1914, and January 1, 1915, and soon thereafter were tested. If anything, therefore, the seasonal effects should have caused the samples from the humid soils to be somewhat depressed in nitrifying efficiency, but they show themselves superior as a class to the arid soils in the nitrification of soil nitrogen. In view of this fact and of the opportunity offered for the comparison of a variety of seasonal effects in both arid and humid soils, great significance can not be attached to the influence of the seasonal variations, which are characteristic of nitrification determinations, on the validity of the results.

The quantity of dried blood employed in the cultures in which that material was tested will next be considered. It has recently been pointed out (5) for dried blood that the nitrification of small quantities may proceed normally in certain soils, as has also previously been shown to be true for calcium cyanamid and for goat manure (7), whereas large quantities would not only permit no nitrification but would actually induce losses of nitrate from the soil. In comparative studies like those described previously the absolute values obtained are not significant except as side issues, provided all classes of soils tested are treated alike.

Such like treatment has, of course, been accorded all the soils. It would therefore seem that the figures are illuminating if not exactly for the use of dried blood in general, at least for 1 per cent of dried blood as the nitrifiable material. Of course, the possibility still remains that with 0.05 per cent or 0.1 per cent of blood, instead of the 1 per cent employed in the cultures, the results in the blood series may have favored the arid and not the humid soils, as they did in these experiments. That, however, is quite unlikely, since it is unreasonable to suppose that smaller quantities of dried blood would have been nitrified with less vigor in the humid soils than the large quantity employed. Except, therefore, as it might have changed absolute values, the procedure in the use of dried blood appears to be no factor in the comparison here made between the powers of humid and of arid soils to nitrify dried-blood nitrogen.

The depression of nitrification in most arid soils by 1 per cent of blood in nitrification experiments was briefly explained by the senior author (6, 7) on the following hypothesis: Ammonification of dried blood proceeds very rapidly. Ammonia is poisonous to the nitrifying organism. If a soil has a large internal surface for adsorption, as, for example, in the presence of large quantities of decaying organic matter, in organic colloids, or a similar material, the ammonia produced by the process of ammonification is quickly adsorbed and removed from harmful action on the nitrifying bacteria. If the contrary is true of the internal surface of soils, such as would obtain in sands poor in humus or in closely packed soils of finer texture, the ammonia given off in the ammonification of dried blood would be toxic to the nitrifying bacteria. This would take place by a direct toxic effect and also by the depressing effect on nitrification of large quantities of soluble organic matter in the soil solution introduced through the solvent effects of ammonia on the dried blood. Very few arid soils have been found incapable of nitrifying 1 per cent of blood which did not also yield a very dark soil solution, indicating the active dissolution of the organic matter present. Very few such soils, moreover, have been noted which do not give off a strong odor of ammonia during the period of incubation. That both dissolved organic matter in the medium and free ammonia deter or inhibit the process of nitrification by pure cultures has been proved for solution cultures by Winogradsky's experiments (14). That the same is true for soils in the presence of mixed cultures has not been known, however, until now. Since the hypothesis was first formulated, the writers have carried out experiments, not possible of description here, which clearly point to the highly toxic nature of relatively small quantities of ammonia to the nitrification of dried blood in soils which otherwise transform the nitrogen of that material into nitrates without difficulty. It is further gratifying to note that Hutchinson (4) has arrived at a similar result to that which the writers have obtained on the toxicity of ammonia to the process of nitrification.

If the hypothesis briefly explained is correct, the difference in the behavior of the humid and arid soils toward the nitrification of dried-blood nitrogen is at once explicable, since the amounts of organic and inorganic colloids are usually much larger in the humid than in the arid soils and would act toward the ammonia produced from dried blood as above explained. Even if it should prove desirable to use smaller quantities of dried blood in cultures to determine its availability in arid soils, the method used heretofore may serve as an excellent means for the comparison of groups of soils and as an index to the soil's internal surface and its status with reference to colloid content.

In connection with this discussion it is cogent to refer to the results obtained by Sackett (10), showing that Colorado soils are superior in nitrifying power to 22 soils from localities outside of that State. These results would seem to imply that for some reason Colorado soils are in general superior in nitrifying efficiency to other soils. A comparison of Sackett's data with the writers', however, does not bear out such an implication when the nitrification of the soil's own nitrogen and not that of fertilizer nitrogen is considered. Thus, for example, in the Pasadena area out of 33 soils about 45 per cent produced 7 mgm. or more of nitrate in 100 gm. of soil in a month's incubation period. Of the 23 Colorado soils tested by Sackett only 21 per cent of such soils were found; yet the average total nitrogen content of the Pasadena soils is probably below that of the Colorado soils and Sackett's incubation period was six weeks and the writers' only four weeks. When a relative instead of an absolute basis of comparison is used, similar results are obtained in other California areas. But the humid soils outstrip the Colorado soils even farther than the Pasadena soils and show clearly a very superior nitrifying power as a class for the soil's own nitrogen to that possessed by the Colorado soils.

So far as the nitrification of sulphate-of-ammonia nitrogen is concerned, the Colorado soils do seem to be superior to other soils if the soils chosen by Sackett are fairly representative of Colorado soils. They are, however, to be considered as a class only slightly superior to the Riverside and Pasadena soils when it is considered that we employed a much shorter period of incubation, much less sulphate of ammonia, and did not inoculate our soils with fresh infusions. In fact, it appears now that an equal comparison of the Riverside or Pasadena soils with the representative Colorado soils would probably show them to be very similar in respect to nitrifying powers for sulphate of ammonia. While it would be difficult to establish any fixed criterion, it would seem, however, that the soil's own nitrogen would for all ordinary purposes best subserve the purposes of soil fertility. If such a criterion is adopted, then the Colorado soils as well as the arid soils of California can not only be said not to be superior in nitrifying power to the humid soils but it is barely possible that they are appreciably inferior in that respect.

Attention must be called to the fact that the classification for purposes of discussion of the Pasadena area as a coast valley is not done with any idea of so classifying it permanently. It is merely done to emphasize its closeness to the sea and its greater rainfall and attendant factors which affect many of the soils in that area so as to make them more characteristic of coast than of interior valley conditions under which the Pasadena area would normally be classed.

Several other minor points of interest with respect to the data which have been discussed deserve consideration here. Most of the soils above studied from the South Atlantic States are deficient in nitrogen, and in that respect resemble, for example, the truly arid soils of the Riverside area. Likewise, with respect to the nitrification of nitrogen in dried blood and cottonseed meal, but particularly the former, the South Atlantic soils behave more like the truly arid soils. Their behavior could therefore be accounted for on the hypothesis explaining the behavior of the truly arid soils toward dried-blood nitrogen, taking the low nitrogen content of the soils as an index of the organic matter present and, hence, indirectly of the total internal surface. That, however, aside from the total nitrogen considerations and their indications that climate exerts additional effects on the soil's nitrifying power, is exemplified by the fact that the South Atlantic soils, while behaving toward dried blood and partly toward cottonseed meal like arid soils, stand, with respect to soil nitrogen and sulphate of ammonia, in the position of the truly humid soils. The latter is more easily explicable on the basis of soil acidity with respect to sulphate of ammonia and that of superior moisture conditions with respect to the soil nitrogen. It will be noted that two soils which did not nitrify their own nitrogen well are the Kentucky and Louisiana soils. The first was collected in December and the second in March, which would probably account for the relative inactivity of the nitrifying organisms. With the exception of the last two soils, it will be noted in Table I, Group I, that the South Atlantic soils are far superior in their nitrifying powers for soil nitrogen to the soils of the Riverside area.

It will be noted that the total nitrogen content of soils in connection especially with the nitrification of dried-blood nitrogen has several times been emphasized. This is done to indicate, in a general way, the likelihood of the presence of certain quantities of organic colloids in the soil on the assumption that the soil's nitrogen content bears a more or less intimate relation to the quantity of organic matter present. The latter, moreover, is considered as an indication in its turn of the amount of internal surface contributed to the soil from that source in addition to that present there by virtue of the surfaces of inorganic constituents, including sand, silt, and clay.

SUMMARY

A study was made of the nitrifying powers, under incubator conditions, of about 40 humid and about 150 arid soils. The soil was used as a medium and the forms of nitrogen employed were soil nitrogen, sulphate of ammonia plus soil nitrogen, dried blood plus soil nitrogen, and cottonseed meal plus soil nitrogen. The humid soils were obtained from the different State and Territorial Experiment Stations, one type of surface soil from each one being used in these experiments. The arid soils employed represented the soil types of four typical soil-survey areas in California. The results obtained appear to justify the follow-lowing statements:

(1) The conclusion appears ineluctable that the nitrifying powers of soils of the arid region are no more intense than those of the humid region. This denies Hilgard's (3) teaching to the contrary and confirms the statement of Stewart (11), which was based on more indirect and less extensive evidence.

(2) While indications are not by any means positive now, it is possible that the data of the writers justify the further conclusion that the nitrifying powers of humid soils are greater than those of arid soils. If such a conclusion could be drawn, it would have to be based merely on the nitrification of soil nitrogen and dried-blood nitrogen. The former being the natural source of supply of most of the available nitrogen obtained by crops, it should really be regarded as the most valuable basis for forming a judgment.

(3) Arid soils, on the other hand, nitrify the nitrogen of sulphate of ammonia and cottonseed meal with much greater vigor than do the humid soils. A reversal of efficiency is manifest between the two groups of soils as regards sulphate of ammonia and cottonseed meal, on the one hand, and dried blood and soil nitrogen, on the other.

(4) These results not only throw new light on the question of nitrification in soils of different climatic regions but also tend to confirm the earlier findings of the writers and others on the important relation of climate to soil properties (9).

(5) The foregoing considerations apply to the two groups in general and not to parts of such groups in particular. For example, the Bay and Ukiah soils do not nitrify soil nitrogen as efficiently as the more arid Pasadena and Riverside soils. This may, however, be accounted for by the seasons at which the collection of the soils was made and by the physical condition in which the Bay and Ukiah soils were received.

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IMMOBILITY OF IRON IN THE PLANT

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INTRODUCTION

Work at the Porto Rico Experiment Station on the assimilation of iron by certain plants, including rice (*Oryza sativa*), has afforded results which seem to show that iron is relatively immobile in the plant after it has once been transported to the leaves. In respect to mobility in the plant iron would thus be similar to silicon and calcium and different from nitrogen, phosphorus, potassium, and magnesium, which are generally considered mobile.

These observations on the immobility of iron are chiefly concerned with the nontransference of iron from leaf to leaf under conditions where the plant was insufficiently supplied with iron. That the mobile mineral elements and nitrogen are translocated from leaf to leaf under such conditions seems proved by Schimper (8)[1] as well as by observations on the growth of plants in media lacking one of these elements. The translocation of nitrogen, potassium, and phosphorus from old leaves and stems to the fruiting parts has, of course, been well established by ash analyses of plants during maturation and by direct experiments (6, p. 585–586). This, however, is not different in principle from the translocation of these elements from old to new leaves, as the constituents must in this case also pass from the old leaf into the stem.

The various facts which seem to point to iron being relatively immobile in the plant are given below.

OBSERVATIONS ON RICE GROWN IN NUTRIENT SOLUTIONS LACKING IRON

Rice grown in nutrient solutions without iron is quite different in appearance from rice grown without nitrogen or phosphorus. In iron-free solutions the leaves of the plants commence to die at the top rather than at the base of the plant. The newer leaves that are formed are almost pure white, very thin, and generally wither before the old leaves. The phenomena in rice grown in absolutely iron-free solutions are not so marked as in plants grown for a time with iron and then transferred to an iron-free solution; since either the amount of iron in the seed does not suffice for the needs of the first leaves or it is incompletely translocated, so that even the first two or three leaves formed are yellowish green in color.

[1] Reference is made by number to "Literature cited," p. 87.

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A lot of rice was grown for 13 days in a nutrient solution well supplied with iron and then transferred for 13 days to a solution identical except for the absence of iron. The leaves formed during growth in the complete nutrient solution were dark green, while the leaves that formed in the 13 days after change to the iron-free solution were yellowish green to creamy-white, the old leaves retaining their dark-green color during this change. The chlorosis or lack of green in the newer leaves was obviously associated with a lack of iron and due to a nontransference of iron from the green lower leaves.

Similar phenomena in regard to the appearance of chlorosis were observed by Molisch (4, p. 92) on growing *Cucurbita pepo*, *Helianthus annuus*, *Zea mays*, etc., in iron-free solutions.

The appearance of rice grown in nitrogen or phosphorus-free solutions was quite distinct from that of plants grown in iron-free solutions; the leaves commenced to die from the base upwards, and new leaves were continually formed at the top of the plant. Here there evidently was a translocation of nitrogen or phosphorus from the old leaves to the new, as growth continued after all material in the seed had been exhausted, and new tissue could not have been formed without these elements.

OBSERVATIONS ON PLANTS AFFECTED WITH LIME-INDUCED CHLOROSIS

Experiments at this station showed that the chlorosis of some plants on strongly calcareous soils was due in part at least to lack of iron; at all events appropriate treatment with iron salts cured the chlorosis. Phenomena similar to that observed in rice grown in iron-free solutions were observed with rice and pineapples (*Ananas sativus*), grown in calcareous soils. Rice when not immediately affected with chlorosis showed the chlorosis in the new leaves, even though the old leaves were green. The new leaves formed came out yellowish green to creamy-white and withered completely, the plants dying from the top down, while the lower leaves remained sound and green. Pineapples behaved similarly, in that the chlorosis appeared first in the new leaves, although frequently the lower leaves followed the new ones in becoming chlorotic, until eventually the whole plant became chlorotic.

If iron were mobile in the plant after once being transported to the leaves, evidently the phenomena would have been different. Iron would have been translocated from the old to the new leaves, the scene of most active growth, and the old leaves would have withered or become chlorotic first.

BRUSHING WITH IRON SALTS THE LEAVES OF PLANTS LACKING IRON

Rice plants grown in certain nutrient solutions with a lack of available iron developed chlorosis and were brushed with a 0.2 to 0.4 per cent solution of ferrous sulphate. In the course of this treatment the tips of leaves just emerging from the stalk were brushed. As these leaves grew

out, the part that had been brushed was a normal green, while the lower, unbrushed part of the leaf was strongly chlorotic and remained so until treated with iron. This would hardly have been the case if the iron were mobile in the leaf tissue. Of course, if a great excess of iron had penetrated the epidermis, it might have been translocated to other parts of the leaf.

The inefficiency of spraying the leaves with iron salts, as a means of curing the chlorosis of pineapples or grapevines, is probably partially due to the immobility of iron in the leaves.[1] With pineapples this treatment completely restored the green color to the leaves treated, but new leaves formed after the treatment were chlorotic.

EVIDENCE FROM ASH ANALYSES

Ash analyses of old, young, and withered leaves generally support the view that iron once conducted to the leaf is immobile, although they by no means afford proof. It should be borne in mind, however, in judging many of the old ash analyses that the determination of the relatively small amounts of iron by precipitation as ferric phosphate is not particularly accurate. Also the accuracy of many results where iron was not the chief element sought may well have been affected by contamination. The writers have found the colorimetric potassium-sulphocyanate method, either in usual form or as modified by Stokes and Cain (9), more accurate than the usual method.

Czapek in his compilation states that old leaves as a rule contain more iron than young ones (1, p. 800). The work of Fliche and Grandeau on the composition of leaves of different trees at various stages of growth supports this (3, p. 487). Analyses made at the Porto Rico Experiment Station of leaves from 1-year-old rough-lemon trees (*Citrus limonum*) show the lower or older leaves to be higher in iron than the young leaves. The lower and upper leaves of plants from four different soils were sampled. The percentages of iron in the dry substance are given in Table I.

TABLE I.—*Percentage of iron in young and in old rough-lemon leaves*

Soil No.	Age of leaves.	Iron (Fe_2O_3) in dry substance.
		Per cent.
I	Young	0.017
I	Old	.044
II	Young	.021
II	Old	.039
III	Young	.012
III	Old	.025
IV	Young	.014
IV	Old	.023

[1] It is significant that with certain kinds of chlorosis of grapevines, treatment of cut stems with the iron salts (method of Rassiguier) has proved more efficacious than spraying the foliage.

With respect to the accumulation in old leaves, iron is similar to silicon and calcium, which also seem immobile in the plant when once transported to the leaves. Young leaves are generally relatively higher in the mobile elements than old leaves. This parallelism, however, really affords little proof, as the relative amount of the different ash constituents present in young and old leaves is probably governed more by the function than the mobility of the elements. Also the accumulation of iron in the old leaves of plants well supplied with iron may merely show that a functioning leaf has a continual need of iron. As iron is in some way associated with the formation of chlorophyll and as chlorophyll is apparently undergoing a continual formation and destruction under the influence of light (2, p. 193; 5) and enzyms (10, p. 746), one can conceive of a physiological necessity for an accumulation of iron in old leaves.

The most that can be said concerning the evidence of ash analyses is that the results are such as one would expect if iron were immobile after being located in the leaf.

DISCUSSION OF RESULTS

The only references to the mobility of iron in the plant which the authors have found in the literature are a discussion by Sachs (7) and a statement by Pfeffer that "in a starved green plant, as well as in a fungus, the iron and potassium may be removed from the older dying organs and transferred to the younger growing parts so that the growth may not immediately cease" (6, p. 417). No data or reference are given in support of this statement. While the movement of potassium under such conditions is generally recognized, the same can hardly be said of iron.

Lack of information on the movement of iron is partially due to the fact that iron has ordinarily been considered of so little interest in plant nutrition as to be disregarded in ash analyses. Also, as already pointed out, the usual method of determining iron in plant analyses is probably not sufficiently accurate to show significant changes.

Sachs, in his interesting discussion of the chlorosis of various plants grown under garden conditions (on a calcareous soil), points out the apparent slowness with which iron moves in plants (7).

From the data presented it is not intended to assert that the non-translocation of iron from leaves is an absolutely general rule for all plants, since the foregoing observations were based chiefly on rice and pineapples.

Various observations on rice and pineapples grown with insufficient iron seem to show that iron after once being transported to the leaves is immobile.

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EFFECTS OF NICOTINE AS AN INSECTICIDE

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INTRODUCTION AND EXPERIMENTAL METHODS

The pharmacological effect of nicotine ($C_{10}H_{14}N_2$) on the higher animals is well understood, but there is practically nothing known about the pharmacological effects of nicotine as an insecticide. Owing to the high cost of nicotine, it is desirable to have a substitute for this insecticide. Before being able to discover, if possible, such a substitute, it is first necessary to ascertain how nicotine affects insects.

In the investigation herein recorded two chief objects have been kept in view: (1) To determine the physiological effects of nicotine as an insecticide, and (2) to trace the nicotine into the insects after it has been applied to them. A brief account of the pharmacological effects of nicotine on other animals and the views pertaining to the physical and chemical effects of nicotine on the cells are also given.

Owing to the small size of the insects utilized in the experiments the usual method of procedure employed by pharmacologists could not be used, because it was impossible to operate on living insects in order to ascertain what tissue is vitally affected by nicotine. Consequently the behavior of the insects treated with nicotine was compared with the behavior of normal and untreated ones; and immediately after the treated ones had died, they were fixed in a fluid containing a nicotine precipitant. By this means the nicotine was precipitated wherever it had gone into the insects; and after making microscopical sections from these insects, it was not a difficult task to trace the precipitated nicotine.

Shafer (20, 21),[2] from the standpoint of a physiological chemist, carried on investigations to determine how contact insecticides kill. He did not

[1] The writer is grateful to the following persons: To Mr. A. F. Sievers, Chemical Biologist in Drug-Plant and Poisonous-Plant Investigations, for extracting pure nicotine from a commercial nicotine material and for verifying the percentage of nicotine in a sample of 40 per cent nicotine sulphate; to Dr. D. E. Jackson, of the Department of Pharmacology, Washington University Medical School; and to Mr. O. D. Swett, Assistant Professor of Chemistry in George Washington University, for reading and criticizing the manuscript of this paper.

[2] Reference is made to Literature cited, pp. 120-121.

experiment much with nicotine and did not endeavor to ascertain what tissue is vitally affected by any particular insecticide, but he seems to infer that nicotine affects the cells chemically in the same way as do the other contact insecticides. Even if nicotine has no therapeutic use, it is classified as a poisonous drug; and for this reason the investigations in which it is used give the best results when it is considered from the standpoint of toxicology.

PHYSIOLOGICAL EFFECTS OF NICOTINE

At the outset it was decided to select one of the most specialized insects and to feed it nicotine so that the results might be compared to those previously obtained after administering nicotine to certain higher animals. Although nicotine as an insecticide is rarely used as a stomach poison, nevertheless the experimentation was begun with this phase of the work. Since pharmacologists have determined that, as a rule, nicotine, regardless of how it is administered, has practically the same general effects, it seems logical that nicotine as an insecticide will also have practically the same effect, regardless of how it is applied.

I.—NICOTINE AS A STOMACH POISON

Since the writer, during the past four years, has made a special study of the behavior of the honeybee, and as the honeybee is one of the highest forms of insects, it was first selected for making a special study of the physiological effects of nicotine on this class of animals.

(*a*) BEES FED PURE NICOTINE

To avoid the complications which often arise when a drug composed of more than one constituent is administered, pure nicotine was fed to bees in the following manner: Honey and pure nicotine were thoroughly mixed in the proportion of 1 part of nicotine to 100 parts of honey. Ten c. c. of this mixture were poured into a small tin feeder covered with parallel pieces of wire; then the feeder and contents were placed inside a triangular observation case, previously described by the writer (17). Fifty bees (guards) were next introduced into this case. On account of the faint nicotine odor emitted from the mixture of honey and nicotine, the bees did not eat the food readily. To be certain that the bees had eaten some of this poisoned food before they died, the honey stomachs of several dead bees were examined. It was found that each stomach contained more or less honey, and this was certainly not eaten before the bees were put into the case, because the honey stomachs of guards never contain honey. Testing this supposedly poisoned honey for the presence of nicotine by using alkaloidal reagents, such as silicotungstic acid and phosphomolybdic acid without first attempting to isolate the alkaloid means nothing, for these reagents also precipitate honey and many other

organic substances. This test did not appear sufficiently significant to warrant the expenditure of more time.

The 50 bees lived from 10 hours to 72 hours, with 33 hours as an average, whereas 50 bees fed honey containing no nicotine lived 8 days, on an average. To facilitate description, the behavior of the bees dying of nicotine poisoning may be divided into three stages, and since nicotine kills the higher animals by paralysis and since, as will be shown, it kills insects similarly, the words "paralyze" and "paresize" may be used from the outset. The word "paresis" means partial motor paralysis, while the word "paralysis" includes both motor and sensory paralysis.

First stage.—Shortly after being poisoned, bees become more or less inactive and are seldom seen eating. They "pay little or no attention" to hive mates or to strange bees and never attack the latter. They soon become stupid, and from then on their behavior is quite abnormal. All their senses are perhaps benumbed, for they do not offer to attack bees carrying foreign hive odors, and they are not very sensitive to mechanical stimuli of any kind. A little later one or both, but usually one, of the hind legs becomes partially paralyzed (paresized), and thereafter they are of little use. Or the front legs may be stricken partially or totally with motor paralysis at the same time, but occasionally the middle legs may be similarly affected before either of the other two pairs is stricken. The wings seem to be paresized before the legs are affected, because a stupid bee removed from the case is able to walk normally and can vibrate its wings, but can not lift itself from the table. Whenever the motor paralysis has not extended further than to paralyze partially the wings and to paralyze totally only one pair of legs, the bees in almost every instance recover when removed from the case to fresh air and when given pure honey. They eat the honey readily and soon throw off their stupor, and the paresized wings and legs soon recover so that after half an hour the bees are again able to fly.

Second stage.—Soon after one pair of legs is stricken, all three pairs and the wings become paresized. During this stage bees act somewhat like a man intoxicated with alcohol. They walk in a staggering manner, drag the paresized legs, and frequently fall down, but never walk upright in the normal manner. Sometimes all three legs on one side may be affected totally by motor paralysis, while on the other side one or more legs may not be stricken. In such a case as this, the bee lies flat on its thorax and abdomen and turns in a circle. Often a bee falls over on its side or on its back and can not get up. Sometimes the middle and hind legs are totally stricken with motor paralysis, while the front legs are apparently not affected. In this case the bee crawls along by dragging its abdomen. A little later when all the legs and wings are affected totally by motor paralysis, the bees are entirely helpless. If removed from the case at this instant, a bee thus paralyzed is still able to extend its tongue and to eat honey, but a few moments later the mentum

becomes paresized, and the tongue can no longer be extended. Subsequently the mandibles and antennæ are stricken.

THIRD STAGE.—At the beginning of this stage the bees are apparently dead, except that an occasional twitching of a tarsus or a slight movement of the end of an antenna or of the abdomen may be seen. Sometimes an abdomen passes through a series of convulsions, and occasionally a small amount of feces is voided; in one instance a small amount of liquid was seen issuing from the mouth.

If the behavior of bees dying of nicotine poisoning is interpreted in the same way as is interpreted the behavior of higher animals likewise poisoned, it seems that nicotine as a stomach poison really kills bees by motor paralysis, and that the paralysis travels along the ventral nerve cord from the abdomen to the head, first affecting the abdominal and thoracic ganglia, then the subesophageal ganglion, and last the brain. Considerable light is thrown on this point in the portion of this paper dealing with the tracing of the nicotine from the time it is applied to the time it reaches the nervous system. Having decided that nicotine kills insects by paralysis, we shall now consider the effects of nicotine as an insecticide when applied in practical work.

2.—NICOTINE SPRAY SOLUTIONS

The following results are not meant to test the efficiency of any of the commercial nicotine spray materials, or of even various dilutions of them, but merely to determine how nicotine affects insects when it is applied as in practice.

(*a*) APHIDS DIPPED INTO SOLUTION OF PURE NICOTINE

Carolina poplar leaves bearing many aphids (*Aphis populifoliae* Davis) were dipped into a solution of pure nicotine (1 : 100). At once the aphids began to exhibit an abnormal behavior and soon showed signs of dying. Half an hour later all of them were dead.

(*b*) APHIDS SPRAYED WITH SOLUTION OF PURE NICOTINE

Many more aphids on Carolina poplar leaves were sprayed heavily with the above solution. Half an hour later nearly all the aphids were dead, every one being dead 15 minutes still later. Before being sprayed, these aphids were quiet and seldom moved from place to place on the leaves. They usually stood on the first two pairs of legs, with the hind pair of legs and abdomen high in the air and with the beaks stuck into the leaves. Occasionally an aphid elevated its abdomen higher into the air and simultaneously moved its body sidewise in a jerky manner. The legs and antennæ were moved little, and no liquid was seen issuing from the cornicles or from the anal openings. Immediately after being sprayed, these same aphids lay flat on the leaves, apparently dead.

They were covered more or less with white "wool," which was less conspicuous after being wetted by the spray. Five minutes later the aphids stood up, began to move their legs, and most of them were comparatively active for a few moments. They removed their beaks from the leaves, moved about considerably by lifting the legs nervously, and their peculiar jerky movements became more conspicuous. Later they were more quiet and the legs became paresized, the hind legs being affected first, the middle legs next, and the front legs and antennæ last. At this stage the hind legs generally are totally stricken with motor paralysis. When paresized, many of the aphids fell from the leaves, and for a few moments they seemed to be recovering from their stupor; but they finally died. However, all of those that fell from the leaves lived several moments longer than those that remained on the leaves. When almost inactive, the aphids fell either over on their sides or on their backs and were completely helpless. The last signs of life were twitchings of the tarsi and slight movements of the antennæ. Before death, the bodies of the aphids appeared perfectly dry. When dead, the legs are usually folded and are stiff. During the various stages of paralysis, it was common to see small drops of clear and dark fluids issuing from the cornicles and anal openings.

(*c*) INSECTS SPRAYED WITH SOLUTION OF NICOTINE SULPHATE

Many aphids on leaves of the Carolina poplar (*Populus deltoides*) were heavily sprayed with a solution of nicotine sulphate, made in the proportion of 1 ounce of the nicotine sulphate to ½ gallon of water, this being 1 part of the insecticide to 64 parts of water, which is 12½ times as strong as recommended for the more resistant sucking insects, such as the black aphis and woolly aphis. This nicotine sulphate is guaranteed to be at least 40 per cent nicotine, and the analysis of this sample showed that it contained a fraction more than 40 per cent. Four hours after being sprayed, all these aphids were apparently dead.

In practical work nicotine as an insecticide is rarely used for caterpillars and probably never in the form of spray for the imagoes of coleopterous and hymenopterous insects, but it was desirable to ascertain how nicotine affects various kinds of insects and to obtain material for the study of the tissues after the insects had died of nicotine poisoning. For this reason various kinds of insects were heavily sprayed with the above solution. An hour elapsed before the large caterpillars of the catalpa sphinx (*Ceratomia catalpae* Bdv.) died; however, a much weaker solution (1 : 1,200) apparently killed the small caterpillars (6 to 10 mm. in length) of the same moth in five minutes, and the same was true of an extract made of powdered tobacco and water (50 gm. of tobacco boiled in 1,000 c. c. of water). The stronger solution of the nicotine sulphate quickly killed the small caterpillars of *Atteva aurea* Fitch, and of *Datana* sp., but it was not so effective on the larger larvæ of the lesser wax moth

(*Achroia grisella* Fab.) and on bagworms, larvæ of *Thyridopteryx ephemeraeformis* Haw. Of the four adult blister beetles (*Epicauta pennsylvanica* DeG.) sprayed, only three died, and worker bees could not be killed by spraying them.

From the foregoing it is thus seen that there is considerable difference in the responses of various insects to nicotine spray solutions. The youngest and smallest individuals of any given species always succumb first and some imagoes, such as bees, can not be killed at all.

3.—NICOTINE AS A FUMIGANT

The following apparatus was devised: To the neck of a 50 c.c. retort supported on a ring stand was connected a piece of rubber tubing 12 inches in length, with its free end projecting into a battery jar 9 inches in diameter and 12 inches in height. The jar was covered with a piece of glass.

(*a*) APHIDS FUMIGATED WITH PURE NICOTINE

Carolina poplar leaves bearing many aphids (*Aphis populifoliae*) were supported in a bottle, and the bottle with its contents was placed inside the battery jar so that the aphids did not touch the sides of the jar. Twenty-five c. c. of pure nicotine were poured into the retort, which was then heated gently. The free end of the tubing was removed from the battery jar, and the heat was still applied. Brownish fumes soon arose from the nicotine; they immediately condensed upon striking the upper, colder portions of the retort, which soon became too warm to condense them. Other fumes then passed into the neck of the retort, where they were likewise immediately changed into liquid, which ran in little streams back into the retort. The rubber tubing was next warmed by the fumes. As soon as drops of the liquid ceased to fall from the free end of the tubing the fumes were passing freely from this end. The tubing was then inserted into the battery jar. At once the aphids began to squirm, and the jar was soon filled with dense fumes. At this instant the burner was removed from under the retort, whereupon the fumes began to condense. A little later small streams of the liquid ran down the sides of the jar, and small drops collected on the underside of the glass cover. The leaves and the aphids seemed to be covered with a fine spray.

So far as could be observed through the dense fumes, the behavior of these aphids was similar to that of sprayed aphids. Before dying many of them dropped from the leaves. Most of them appeared dead within three minutes after the introduction of the fumes; two minutes later still all of them were dead.

The preceding mode of procedure has been described in detail in order to make evident the ease with which the liquid can be applied by fumigating to cool surfaces with which the vapors may come in contact. Since the temperature of insects is practically the same as that of the

air surrounding them, it seems evident that the nicotine fumes would be condensed upon striking the integuments and tracheal walls of the insects fumigated.

The preceding experiment was repeated by using aphids (*Aphis rumicis* L.) on nasturtiums. Small pots containing these plants were placed inside the battery jar. Five minutes after introducing the fumes all the aphids were dead.

(*b*) INSECTS FUMIGATED WITH 40 PER CENT NICOTINE-SULPHATE SOLUTION

The preceding experiments were repeated by fumigating the following insects with a 40 per cent solution of nicotine sulphate: Aphids on nasturtiums (*Tropaeolum majus*) and those (*Myzus persicæ* Sulz.) on potato plants (*Solanum tuberosum*), coccids (*Orthezia insignis* Dougl.), fall webworms (caterpillars of *Hyphantria cunea* Dru.), larvæ of potato beetles (*Leptinotarsa decemlineata* Say), imago house flies (*Musca domestica* L.) and worker honeybees (*Apis mellifica* L.).

The aphids and coccids died a few minutes after the introduction of the fumes, and the plants which bore them were also affected considerably by the fumes. The leaves on the potato plants soon wilted, and some of them finally turned brown. They emitted a comparatively strong nicotine odor for several days, and even a very faint nicotine odor was perceptible 15 days after the plants were fumigated.

The fall webworms and potato-beetle larvæ (two-thirds grown) were not so easily killed, although after being confined for a period of 15 or 20 minutes in dense fumes, they die. While dying, the caterpillars wriggle about considerably and exude a yellowish fluid from the mouths. Bees die in the same length of time, but house flies do not succumb so readily. Bees, when apparently dead, often revive if they are removed from the jar to fresh air.

The preceding experiments indicate that nicotine as a fumigant kills insects by paralysis and that part, if not all, of the fumes, which strike the integuments and which pass into the tracheæ of the insects, are condensed before they enter the various tissues. On page 110 it is shown that the nicotine never passed far from the tracheæ into the tissues. This supports the view that nearly all of the fumes in the tracheæ were changed into liquid which did not pass readily through the tracheal walls. It is also seen that the most delicate insects yield first to nicotine fumes.

4.—NICOTINE ODOR AND VAPOR

To determine the effects of nicotine odor on insects, leaves were either sprayed with or dipped into nicotine spray solutions. Their stems were then inserted into bottles of water which were placed in the sun or in front of an electric fan. The leaves were always left in the current of

air from the electric fan for an hour, at the end of which time they were perfectly dry, but still emitted a very faint odor of nicotine. A slightly longer time in the sun was required before they became perfectly dry. Each bottle with its contents was placed inside a battery jar 5 inches in diameter by 11 inches in height. Normal and untreated insects were then removed with a camel's-hair brush from other leaves to the leaves in these bottles. A glass cover was placed over each bottle, and the insects were observed at regular intervals.

To ascertain the effects of nicotine vapor on aphids and bees, the insects were either inclosed in a battery jar with nicotine spray material below them or with the spray solutions placed in watch glasses or on the leaves near the insects in the open.

(*a*) ODOR FROM SOLUTION OF PURE NICOTINE

Carolina poplar leaves were dipped into a solution of pure nicotine (1:100), placed in the current of an electric fan for an hour, and were then arranged as already described. At 11 o'clock aphids (*Aphis populifoliae*) from other leaves of the same tree were transferred to the leaves treated with the nicotine solution. At 4.30 o'clock the aphids were slightly stupid. The next morning all of them were dead. Not one of the aphids used as controls died.

One day aphids were killed by being placed in vials which a week before had contained some of the nicotine solution. These vials after having been used had not been washed, and a week later two of them were unintentionally used for collecting aphids in the greenhouse. By the time a dozen aphids had been put into each vial and closed with stoppers which had also been used a week before, most of the insects were dead, and the remainder of them were in the last stage of paralysis. An examination showed that the vials still gave off a very slight odor of nicotine.

(*b*) VAPOR FROM PURE NICOTINE

At 10.30 o'clock a large Carolina poplar leaf bearing many aphids was put into one of the battery jars. A small beaker containing 5 c. c. of pure nicotine was also placed inside the jar about 5 inches below the leaf. At 12 o'clock a few aphids were stupid; at 1 o'clock several were dead; at 4.30 most of them were dead; the next morning all of them were dead.

A few cubic centimeters of a pure nicotine solution (1 : 100) were poured into each of seven watch glasses. A small wire screen was laid on top of each watch glass so that it did not touch the nicotine solution. Several cabbage aphids (*Aphis brassicae* L.) were then placed on each wire screen. The smallest aphids died within 10 minutes, the medium-sized ones within 16 minutes, and the largest ones within 22 minutes.

At 11 o'clock the upper surface of a large dock leaf (*Rumex* sp.) was sprayed heavily with the nicotine solution (1 : 100) in the greenhouse. A comparatively large nasturtium leaf was placed directly over and one-half inch above the dock leaf. The under surface of each leaf bore many aphids. At 2 o'clock all the aphids on the underside of the nasturtium leaf were dead, while none on the dock leaf apparently had been affected.

(*c*) ODOR FROM EXTRACT OF POWDERED TOBACCO

Catalpa leaves were sprayed heavily with the extract of powdered tobacco described on page 93. After these leaves had become perfectly dry in the sun, they still emitted a faint nicotine odor. At 1 o'clock many small caterpillars (6 to 10 mm. in length) of the catalpa sphinx were then tested by being placed inside battery jars in the manner already described. At 1.10 o'clock a few of them were dying; at 2.25 four-fifths of them were dead. These had not eaten of the leaves. The remaining ones did not die until three days later; they had eaten the leaves to a limited degree.

The preceding experiment was repeated twice by using large fall webworms on mulberry leaves (*Morus* sp.) that had been submerged for two minutes in the extract and had been dried in the current of air from the electric fan. The first lot of caterpillars ate the leaves readily and apparently were not affected, but a third of the second lot was dead the following morning, after having slightly eaten the leaves.

(*d*) ODOR FROM SOLUTION OF NICOTINE SULPHATE

The experiment just preceding was repeated twice by using a solution of nicotine sulphate (1 : 1,200). The results obtained with the first lot of fall webworms showed that only one caterpillar was killed, but the leaves were not much eaten. Relative to the second lot, the leaves were not eaten at all. Two hours after being placed on the leaves, many of the caterpillars became stupid and a few showed signs of dying, but were not found dead until the following morning. Two days later still all of them were dead.

The preceding was repeated by heavily spraying catalpa leaves with a much stronger solution of nicotine sulphate (1 : 64) and by using small caterpillars (6 to 10 mm. in length) of the catalpa sphinx. At 12.52 o'clock these leaves had become perfectly dry in the sun; at 12.56 a few of the caterpillars acted as if dying; at 2.25 all of them were dead. The leaves gave off a slight nicotine odor and had not been eaten.

The preceding was repeated by dipping Carolina poplar leaves into the same solution and by using the aphids removed from these leaves after the latter had been dried in the current of air from the electric fan. The following morning most of these aphids were dead.

(*e*) VAPOR FROM A 40 PER CENT NICOTINE-SULPHATE SOLUTION

Fifty worker bees in an observation case were introduced into a large battery jar. A small quantity of a 40 per cent nicotine sulphate solution in a Petri dish 10 cm. in diameter was placed inside the jar 8 inches beneath the case of bees. During all the following day the bees remained more or less inactive and appeared slightly stupid. The next day following they were still slightly abnormal in behavior, but none died.

The upper surfaces of large dock leaves were heavily sprayed with two solutions of nicotine sulphate (1 : 100 and 1 : 500) in the greenhouse. The aphids on the under surfaces of these leaves apparently were not affected by the vapor from either solution. On the other hand, when a small amount of the stronger solution was placed on the under surfaces of nasturtium leaves near the aphids but not against them, most of the insects were found dead three hours afterwards. When a piece of cheesecloth wet with the weaker solution was placed an inch beneath the branches and leaves of a nasturtium, a few of the many insects on this plant were found dead.

In view of the results of all the preceding experiments in which the spray solutions had been evaporated, it may be argued that many of the insects died of nicotine poisoning by eating the leaves which had previously been treated with nicotine solutions. That these leaves still emitted a faint odor of nicotine indicates that their surfaces still bore many traces of the alkaloid. It is also probable that some of the nicotine passed into the tissues of the leaves. Since some of those insects that did eat the leaves died so quickly after being placed inside the jars, it does not seem logical that they died primarily from the effects of nicotine as a stomach poison, because a small amount of nicotine as a stomach poison acts slowly. In view of the preceding reasoning and since some of the insects did not eat the leaves at all, it seems safe to say that most of them were killed by the odoriferous particles of the nicotine passing into the tracheæ. In all of those experiments in which the insects were subjected to nicotine vapor, although they did not actually touch the nicotine solutions, there can be no doubt that the vapors killed the insects; and it is also probable that the vapors passed into the tracheæ and killed by paralyzing the nervous system. These experiments demonstrate that nicotine spray solutions are not necessarily contact insecticides, although they are more effective when actually used as such, for by this means the insects are constantly brought near the vapor under the most favorable conditions.

TRACING NICOTINE TO TISSUES

Owing to the small sizes of the insects used, it was not considered possible to operate successfully on live individuals in order to determine what particular tissue is vitally affected when nicotine is used as an insecticide. Drawing conclusions solely from the behavior of the insects dying of nicotine poisoning, the author states in the preceding pages that they die of

paralysis. Since paralysis is an affection of the nervous system, it still remains to be shown that nicotine applied as an insecticide reaches the nervous system and how it affects the nerve cells. The following pages deal with this portion of the work. Many difficulties were encountered, and the experiments performed to determine how nicotine affects the nerve cells gave no definite answer to this question. The latter phase of this subject is presented mostly by giving a brief discussion of the various views pertaining to the physical and chemical effects of drugs on cells.

Tracing the nicotine into the various insect tissues was accomplished by precipitating this alkaloid immediately after it had killed the insects and then by carefully studying the microscopical sections made from the insects thus tested. A study of this nature involves considerable technique and many precautions in making sections, because two objects instead of one must be successfully accomplished at the same time. It is an easy matter to obtain good sections of most larvæ and soft-bodied insects under ordinary conditions, but it was found quite difficult to obtain good sections and at the same time not to lose the precipitates held in the tissues while the slides were being run through the various reagents. This is appreciated when we consider the solubility of various substances in the clearing oils, in the alcohols, and in water.

In addition to the difficulties enumerated above, there are still three more to be considered: (1) After a certain period has elapsed following death as a result of having been treated with nicotine, the tissues of the insects were unusually abnormal upon fixation. As soon as life is extinct, and probably a short time before, the cells gradually change from normal to abnormal ones. This was particularly noticeable when small caterpillars were sprayed with solutions of nicotine. A short time after death they turned brown and the tissues were found to be more or less disintegrated. For this reason it was always necessary to fix the insects just before the last signs of life had disappeared in order to avoid mistaking post-mortem changes in the cells for physical ones caused by the nicotine. (2) On the other hand, if nicotine really causes physical changes in the cells of insects, these changes are always masked by the large physical ones caused by the fixing reagents. (3) It is often difficult and sometimes impossible to distinguish the precipitated insecticide inside the tissues from the coagulated constituents of the cells caused by the fixative. Fischer (8) regards the coagulation of these constituents, which really constitutes fixation, as a true precipitation, but of course it is a milder form. The coagulated particles are, nevertheless, frequently as large and sometimes larger than the precipitated ones.

I.—TRACING COLORED LIQUIDS INTO INSECTS

Before determining whether or not nicotine spray solutions as applied under practical conditions reach the tissues by passing through the spiracles, many preliminary experiments were performed to ascertain

whether water and nicotine solutions, applied under the most favorable conditions, are able to enter the spiracles, mouths, and anal openings of various insects. In order to follow these liquids, they were colored with various stains.

(*a*) ABILITY OF COLORED LIQUIDS TO ENTER THE SPIRACLES AND ALIMENTARY CANAL

A small quantity each of water and of pure nicotine solution (1:100) was colored with each of the following aqueous stains: Carmine acid (Grübler's Carminsaur), eosin, gentian violet, Delafield's, Ehrlich's, and pure hematoxylins, methylene blue and safranin. Cabbage aphids were submerged in each of these colored liquids for an hour. After removal from the liquids they were well washed in water, then crushed, and finally mounted on slides in glycerin. The glycerin did not mix with nor scatter the stains. Various parts of the integuments were colored more or less with each stain, but methylene blue seemed to penetrate the integuments the most readily of any of these stains. Each stain seemed to pass into the tracheæ more or less, but the four following ones entered most readily: Carmine acid, gentian violet, methylene blue, and safranin. Of these four, carmine acid proved to be the best. The tracheæ in most of the aphids showed scarcely any of the stain, while those in the remaining ones showed it conspicuously. In these few insects all the larger tracheæ in the abdomen, thorax, and head were stained; and occasionally a stained trachea was traced into a leg. There seemed to be no difference in permeability between the stains dissolved in water and those dissolved in the nicotine solution.

The preceding experiments were repeated by submerging roaches (*Periplaneta americana* L.), croton bugs (*Blattella germanica* L.), house flies, and larvæ of blow flies (*Calliphora vomitoria* L.) for an hour in water and in a pure nicotine solution (1:500), each being colored with carmine acid. The stain was observed in the esophagus and anus of the roaches and croton bugs; in a few of the larger tracheæ and in many of the smaller ones and in the hind gut of the fly larvæ. The house flies were fixed in absolute alcohol, which readily throws down carmine acid.[1] One of the flies that had been submerged in the colored nicotine solution was sectioned, and the sections were placed in xylol alone without being stained, in order that none of the "precipitated" carmine acid might be lost. A study of the sections showed the "precipitated" carmine in several of the larger tracheæ (Pl. 1, fig. A, *pr*). An examination of the other flies showed that the colored liquid had passed into some of the larger tracheæ and into the rectums.

The preceding experiments were repeated by submerging green peach-aphids (*Myzus persicae*) in pure nicotine solution (1:500), colored with

[1] Absolute alcohol does not precipitate carmine acid nor indigo-carmine, but merely throws them out of solution, because they are not soluble in it. For lack of an appropriate term to describe these stains when thrown out of solution the word precipitate in quotation marks may be used.

indigo-carmine (sodium sulphindigotate) for 45 minutes. These insects were fixed in absolute alcohol, which readily throws down indigo-carmine. The resulting "precipitate" is totally insoluble in xylol and absolute alcohol, but its solubility in the other alcohols increases as the water in them increases. For this reason the sections of these aphids were stained in absolute alcohol containing safranin. The blue "precipitate" was common on the outside of the integument, but it was not seen inside the integument anywhere, except occasionally in the larger tracheæ and then usually not far from the spiracles. Plate 1, figure B, represents the "precipitate" (*pr*) observed in two places in the same trachea. This drawing was made from two consecutive sections and shows the most "precipitate" that could be found.

The experiment just preceding was repeated by submerging larvæ of wax moths (*Achroia grisella*) and small nymphs of croton bugs in the above liquid for 30 minutes. A small amount of blue "precipitate" (Pl. 1, fig. C, *pr*) was observed in most of the larger tracheæ of the wax moths and occasionally some (Pl. 1, fig. D, *pr*) in the smaller ones. Each of the sections of the croton bugs, containing parts of the alimentary canal, shows more or less blue "precipitate" inside this tube; but none was observed elsewhere inside the integument.

Thirty worker bees were submerged for 30 minutes each in water and in pure nicotine solution (1 : 500), each liquid being colored with carmine acid. When removed from the liquids, the bees were thoroughly washed in water. The apparently dead bees were then laid on blotting paper in the sun to become dry and to revive from the effects of the liquids. All 30 bees submerged in the colored water revived and were walking about in from 12 to 18 minutes, with an average of 15 minutes, after being placed in the sun. Only 60 per cent of those submerged in the colored nicotine solution revived, and these never became able to fly as did those submerged in the colored water. The time required for them to recover sufficiently so that they could crawl about varied from 45 minutes to 3¾ hours, with about 2 hours as an average.

An examination of the live bees just described showed that the thin chitin between the segments was often colored red and that when a thorax was crushed, the red liquid usually issued from the mouth. When the bees that had been submerged in the colored water were dissected, the stain was seen in 90 per cent of the honey stomachs, in 64 per cent of the ventriculi, in 50 per cent of the rectums, and in 50 per cent of the anal openings and around the stings. When the bees that had been submerged in the colored nicotine solution were dissected, the stain was observed in 45 per cent of the honey stomachs, in only 6 per cent of the ventriculi, never in the rectums, and in 45 per cent of the anal openings and around the stings. The behavior of the bees when placed into the nicotine solution may be used to explain why such a small amount of the colored solution passed into the alimentary

canal. While the bees placed into the colored water struggle and cling to one another for several moments after being submerged, those placed into the colored nicotine solution struggle little and never cling to one another. They seem to be slightly paresized as soon as put into the solution, and perhaps for this reason alone they swallow little of the liquid. Paresis may also be used to explain why the colored liquid is not forced from the honey stomachs into the ventriculi.

That the colored liquids were never seen in any of the tracheæ of the 60 bees submerged demonstrates that the valves guarding the spiracles closed water-tight at the instant of placing the bees into the liquids. That one-half of the bees submerged in the nicotine solution did not swallow any of it indicates that these valves can not be closed air-tight, because there seems to be no way of explaining why the bees were paresized other than by supposing that vapor from the nicotine solution passed the valves and entered the tracheæ. Of course, the vapor might have entered the insects through the mouths and anal openings, but this view is highly improbable, and the liquid had not penetrated the integuments even at the thinnest places.

To determine whether the red liquid passed through the thin chitinous layer of the honey stomachs, several of these organs which were almost full of the red liquid were removed after both ends of a honey stomach had been securely ligatured with thread. Immediately after a honey stomach had been dipped into water to moisten its walls, it was gently rolled on white paper. No red liquid was seen issuing through its walls; nor was any observed on the paper. The same experiment was repeated by using the ventriculi. In this case red liquid was plainly seen to issue from the walls of each ventriculus, and it made the paper red. Thus, it seems that the red liquid usually seen surrounding the viscera of these bees when cut open had not passed through the walls of the honey stomachs, but through those of the ventriculi and probably to a limited degree through the walls of the small intestines and those of the rectums, although it is shown on page 108 that a nicotine solution containing indigo-carmine does not pass through the walls of the small intestine and rectum.

In all the preceding experiments liquids colored with stains have been used. It is probably true that many stains increase the permeability of their solvents and consequently may also increase the ability of the solvents to pass into small openings, such as the spiracles. For this reason coccids (*Orthezia insignis*) were submerged for 30 minutes in a pure nicotine solution (1 : 100) which had not been colored with a stain. They were then fixed for 15 minutes in a mixture consisting of two parts of absolute alcohol and one part of phosphomolybdic acid. To insure the removal of all the phosphomolybdic acid not united with the nicotine and to insure better fixation, the insects were put into a mixture consisting of two parts of absolute alcohol and one part of

glacial acetic acid. After remaining in this mixture for about three hours, they were placed into absolute alcohol for another hour.

Phosphomolybdic acid is one of the alkaloidal reagents, and it precipitates nicotine even in a dilution of 1 to 40,000. It was prepared according to the directions of Autenrieth (1) with modifications as follows: A sodium-carbonate solution was saturated with pure molybdic acid; one part of crystallized disodium phosphate (Na_2HPO_4—$12H_2O$) to five parts of the acid was added and the mixture evaporated to dryness. The residue was fused and the cold melt was dissolved with absolute alcohol. This mixture was filtered and enough nitric acid added to produce a golden-yellow color. The resulting mixture, called "phosphomolybdic acid," was used full strength when mixed with absolute alcohol to serve both as a fixative and as a precipitant. The precipitate resulting from the union of this mixture and nicotine is neither soluble in water nor in any of the alcohols; but for fear of losing some of the precipitate the sections were stained in safranin dissolved in 95 per cent alcohol.

A study of the sections of the coccids treated as described above showed a brownish yellow precipitate inside many of the tracheæ (Pl. 1, fig. E, *pr*), but it was not seen elsewhere inside the integument. In the sections of coccids used as controls no precipitate was seen anywhere. It is thus seen that nicotine solutions containing no stains are able to pass into the tracheæ of coccids that have been submerged in the solution for 30 minutes.

(*b*) ABILITY OF NICOTINE SPRAY SOLUTIONS TO ENTER SPIRACLES

Aphids (*Aphis brassicae*, *A. rumicis* L., and *Macrosiphum sanborni* Gill.), and coccids (*Orthezia insignis*) were sprayed with a pure nicotine solution (1:500), colored with carmine acid, until they were wet with spray. An hour later they were mounted on slides, as described on page 100, and were examined. The spray had evaporated, leaving the red stain adhering to various parts of the integuments. Nearly all of the tracheæ showed no signs of the stain; but a few seemed to be slightly pink, although this kind of an examination is not entirely reliable.

The preceding experiments were repeated by heavily spraying aphids (*Aphis rumicis*) with a pure nicotine solution (1:500), colored with indigo-carmine. The sections were stained as described on page 101. A thorough study of these slides showed that the colored nicotine solution had not passed through the integuments nor into the tracheæ. At only one place was it found that the "precipitate" had lodged in a spiracle (Pl. 1, fig. 6, *sp*); but it was never observed in the tracheæ (Pl. 1, fig. 7, *tr*), nor elsewhere inside the integuments, although it was commonly seen adhering to the outer surfaces of the integuments (Pl. 1, fig. 7, *int*).

Aphids of the same species as just described were heavily sprayed with a pure nicotine solution (1:100), not colored with any stain. These

aphids were fixed with the mixture of absolute alcohol and phosphomolybdic acid and were further treated as described on page 102. No precipitate was found in the tracheæ nor elsewhere inside the integuments.

Some of the caterpillars of the catalpa sphinx (*Ceratomia catalpae*), of *Atteva aurea*, of *Datana* sp., and larvæ of the lesser wax moth, *Achroia grisella*, that had been sprayed with a solution of nicotine sulphate (1:64) were fixed in Carnoy's fluid. This fixative is a mixture composed of equal parts of absolute alcohol, chloroform, and glacial acetic acid, saturated with mercuric chlorid ($HgCl_2$). Mercuric compounds are among the general alkaloidal reagents and the mercury in mercuric chlorid readily precipitates nicotine. The sections of the above larvæ were stained with Ehrlich's hematoxylin and the crystals of the mercuric chlorid remaining in the tissues after the fixative had been washed out were removed by 95 per cent alcohol containing tincture of iodin. The iodin unites with the mercuric chlorid, forming a compound which readily dissolves in alcohol, but iodin apparently has no effect on the gummy precipitate resulting from the union of nicotine and Carnoy's fluid. For this reason, if the spray solution passed into the tracheæ, the nicotine in it should have been precipitated and should not have been affected by the iodin, and all of it certainly could not have been washed out while the slides were being run through the reagents. At any rate, after the sections had been treated with tincture of iodin, no precipitate of any kind was observed inside the integuments of these larvæ. This indicates that a spray solution of nicotine sulphate does not enter the spiracles nor pass through the integument; but this method is not fully reliable, on account of having to remove the crystals of mercuric chlorid.

Since it has been shown that spray solutions, as applied under practical conditions, do not pass into the tracheæ, a study of the spiracles of the aphids, coccids, and larvæ that have been used in the experiments shows that it is practically impossible for aqueous solutions to enter the spiracles. The mouths of the spiracles of all these insects, except the coccids, are guarded by pseudohairs, which are outgrowths from the chitinous linings of the spiracles and by the rims (Pl. 1, fig. J, *r*) turning inward and sometimes downward. The spiracles (Pl. 1, fig. H, *sp*) of the coccids are practically unprotected, while those of aphids (Pl. 1, fig. B, *sp*) bear a few hairs. The small size of these spiracles seems to be the best reason why aqueous solutions can not readily pass into the tracheæ. The hairs guarding the spiracles of some of these insects vary from short, stout ones, as in the larvæ of *Atteva aurea* (Pl. 1, fig. I, *sp*), in the lesser wax moth (Pl. 1, fig. J, *sp*) and in *Datana* sp. (Pl. 1, fig. K, *sp*) to long, stout ones, as in the caterpillar of the catalpa sphinx (Pl. 1, fig. L, *sp*) and in the larva of the Colorado potato beetle (Pl. 1, fig. M, *sp*). The hairs (Pl. 1, fig. N, *hr*) in a spiracle of the fall

webworm are branched, and they nearly close the entrance, while the entrance of a spiracle (Pl. 1, fig. O, *sp*) in the tomato worm (larva of *Phlegethontius sexta* Joh.) is closed by a hairy and porous plate (Pl. 1, fig. O, *p*), which has an oblong opening through its center.

Shafer (20) colored kerosene with Sudan III; and after thoroughly spraying or dipping grasshoppers (*Melanoplus femoratus* Burm.), tomato worms, and aphids into this oil and after dissecting these insects, he found more or less of the red oil in the larger tracheæ. He colored kerosene emulsion and the emulsions of the miscible oils with indigo-carmine and with safranin and found that they also enter the spiracles. Shafer repeated these experiments by treating aphids with creolin emulsion containing indigo-carmine. After fixing the insects in absolute alcohol and after studying the sections he observed plugs of "precipitated" indigo-carmine in the larger tracheæ, which were sufficiently large to close them.

Dewitz (7), in discussing contact insecticides, does not believe that either liquids or powders can enter the spiracles in sufficiently large quantities to cause the death of the insects by suffocation.

Without attempting to apply the physical law governing the surface tension of liquids, the following experiment was performed to determine roughly the surface tensions of water, different solutions of 40 per cent nicotine sulphate, pure nicotine, kerosene, gasoline, and kerosene emulsion. Fresh nasturtium leaves were spread out flat on a table, with the under surfaces upward. With pipettes drops of water, solutions of nicotine sulphate (1:500 and 1:100), undiluted 40 per cent nicotine sulphate, undiluted pure nicotine, pure nicotine solutions (1:500 containing indigo-carmine and 1:100), kerosene, gasoline, and kerosene emulsion were dropped upon the nasturtium leaves. Of all these liquids the surface tension of gasoline was weakest and that of water the strongest; that of kerosene was second weakest, while those of pure nicotine and kerosene emulsion were about equal, but still much stronger than that of kerosene. So far as practical work is concerned, the ability of 40 per cent nicotine sulphate and its solutions and of the two enumerated solutions of pure nicotine to spread over the surfaces of these leaves is about equal to that of water. The drops of each one of these liquids upon striking the leaves form small spheres and are not retained when the leaves are somewhat inclined.

In regard to the evaporation of the solutions of nicotine sulphate and of pure nicotine, the drops of the solutions of pure nicotine evaporated rather quickly while those of the nicotine sulphate did not disappear for some time. The more nicotine contained in the drops of the pure nicotine solutions, the more quickly they evaporated. The evaporation of the drops of the solution of nicotine sulphate (1:500) was about equal to that of the water drops.

From what we know about the relative surface tensions of nicotine solutions, of kerosene, and of various emulsions, it is easily understood why kerosene and the emulsions are able to pass into the tracheæ while the nicotine solutions can not.

2.—TRACING NICOTINE AS A STOMACH POISON TO TISSUES

To obtain material for tracing nicotine as a stomach poison to the tissues and to determine the effects of an extremely small amount of nicotine and of indigo-carmine on bees, the following experiments were performed: 200 drops of pure honey were put into a feeder; 200 drops of honey mixed thoroughly with 40 drops of water colored blue with indigo-carmine were poured into a second feeder; 200 drops of honey mixed thoroughly with 40 drops of pure nicotine solution (1:500) colored blue with indigo-carmine were poured into a third feeder. Each of these feeders, with its contents, was placed inside an observation case, and 50 worker bees were introduced into each case. Before all the bees died nearly all the food had been eaten. Since bees confined in observation cases can not void their feces, the abdomens of these bees became much distended with the blue-green food. The bees that ate the pure honey lived eight days, on an average, while those in the two other cases lived about seven days, on an average, showing that the extremely small amount of nicotine did not affect their longevity, whereas the indigo-carmine seemed to shorten their lives by one day.

The preceding experiments in feeding bees nicotine and indigo-carmine were repeated; and three days later, when several bees showed signs of dying, they were placed into absolute alcohol for two days. The anterior portions of their abdomens, and occasionally the base of a leg, appeared blue-green from the outside. When cut open under absolute alcohol, all the tissues in the abdomen appeared blue-green. A closer examination, however, showed that the alimentary canal was blue, while the other tissues in the abdomen as a rule were pale blue-green, with now and then darker colored streaks running through them. A few muscle fibers and some parts of the chitin were pale blue, and other parts of the chitin were pale blue-green. Under alcohol the tissues in the thorax and head did not appear colored at all; but after being removed from the alcohol and dried, they assumed a pale blue-green color, and occasionally darker colored blue streaks were seen in the muscles and brain. It seems that the indigo-carmine had colored the blood or body fluid pale blue-green and that this fluid in turn had colored all the tissues, but the stain was diluted too much to be "precipitated," except in a few organs.

Parts of the alimentary canal and various tissues were dissected out, and sections were made of them. Sections through the anterior and middle portions of the honey stomach failed to show any blue "precipi-

tate" either in the lumen or in the walls of this organ, but sections through the posterior portion of the honey stomach and other parts of the alimentary canal distal to the honey stomach usually showed more or less blue "precipitate." There was no difference in distribution of the stain, whether or not it contained nicotine, but since the distribution of nicotine only is of interest, the discussion will be confined to the distribution of the stain which formerly contained this insecticide.

Plate 2, figure D, represents the blue "precipitate" as seen in the wall of the lower portion of the honey stomach. The stain seemed to have united with the alcohol as the former was passing through the chitinous layer (*chl*) of this organ. A little "precipitate" was also seen in the muscular layer (*m*) of this organ.

Sections through the anterior portion of the valve of the proventriculus show small particles of "precipitate" in the muscles (Pl. 2, fig. E, *m*), epithelial cells (*ep*), and tracheæ (*tr*).

Sections through the ventriculus show "precipitate" in various places of the epithelium, indicating that the stain was in the act of passing through the wall when it was overtaken by the alcohol. From the location of the blue "precipitate" some of the stain was just ready to pass into the inner ends of the epithelial cells, while some had just entered these cells (Pl. 2, fig. A, *pr*). Other portions of the stain were "precipitated" midway between the inner and outer walls of the epithelium (Pl. 2, fig. B, *pr*), while still other portions were overtaken by the alcohol when they were passing through the outer wall of the epithelium (Pl. 2, fig. C, *pr*). At this location a small amount of "precipitate" was also seen in the transverse muscular fibers (Pl. 2, fig. C, *tm*), indicating that, while most of the stain passed between the muscular fibers, some of it passed into and probably through the fibers.

At only one place was blue "precipitate" (Pl. 2, fig. H, *pr*) observed in the blood (*bl*). This was seen a short distance from the ventriculus near a small trachea (*tr*) and two Malpighian tubules (*mal*), which also contain a little "precipitate." Two particles of this "precipitate" are lying in the outer walls of these tubules, indicating that the stain was passing into these organs when it was thrown down. It was also observed that the trachea contained several small particles of "precipitate."

Many of the Malpighian tubules, particularly those near the honey stomach, small intestine, and rectum, showed no traces of the stain, while those near the ventriculus contained a small amount of it, as represented in Plate 2, figure H, and whereas those against the ventriculus contained large amounts of the "precipitate," as represented in Plate 2, figure G.

The "precipitated" particles in sections through the middle of the ventriculus are more numerous and more compact than in sections through either end of this organ. These sections are never perfect,

and since the tissues were fixed with absolute alcohol, which must have passed into the bees chiefly through the mouths and anal openings, the cells in the epithelial lining of the alimentary canal were not well preserved, so they have been drawn diagrammatically in outline from Snodgrass (22). In sections through the middle of the ventriculus, the "precipitate" is arranged more or less in concentric circles (Pl. 2, fig. J, *pr*). This arrangement is probably caused by the peritrophic membranes being likewise arranged. In the lumen (*l*) and between the concentric circles of "precipitate" the "precipitated" particles are scattered irregularly. In Plate 2, figure J, the epithelial (*ep*) and muscular (*m*) walls have been drawn diagrammatically, showing how the stain probably passes through these walls into the blood where most of it is taken up by the Malpighian tubules. The other parts of this figure were drawn with the aid of a camera lucida.

Since no blue "precipitate" was observed in the epithelium (Pl. 2, fig. F, *ep*) of the small intestine, it seems that the stain did not pass through the walls of this organ, although "precipitate" was easily seen in the lumen (*l*) of the small intestine. Most of the "precipitate" usually occurred in large particles near the center of the lumen, while the inner wall of the epithelium was often lined with a layer of "precipitate" composed of innumerable small particles.

Despite the fragmentary sections of the rectum, a careful study was made of this organ, but no blue "precipitate" was seen in its walls; nevertheless it was quite conspicuous in its lumen (Pl. 2, fig. I, *l*). In the rectum, as well as in the other parts of the alimentary canal, there was considerable "precipitated" material which was not stained. This and the pollen grains were easily distinguished from the "precipitated" indigo-carmine by the blue color of the latter.

The preceding results obtained in tracing nicotine as a stomach poison by means of "precipitating" indigo-carmine is not meant to be conclusive, but merely to point out the possibilities for future investigations along this line. For these results to be conclusive, the nicotine should have been traced without the aid of a stain like indigo-carmine; but owing to the odor from this insecticide bees can not be forced to eat food containing a large quantity of nicotine. For this reason it did not seem possible in preliminary work of this nature to be able to trace an extremely small amount of nicotine without using some comparatively harmless stain with it.

In passing through the walls of the ventriculus, it is scarcely possible that the nicotine and indigo-carmine were separated from one another, and the experiments on page 91 show that nicotine as a stomach poison kills by paralysis. It must therefore be concluded that nicotine in passing through the walls of the ventriculus is not so materially changed as to destroy its effectiveness. That all the tissues, even including the brain, were stained more or less with the indigo-carmine

shows that this substance was widely distributed, and it is also logical to think of the nicotine accompanying the stain wherever it went, except when the stain penetrated hard tissues, such as chitin. In the higher animals nicotine is chiefly excreted through the kidneys, because it is found in the urine soon after it has been administered. Since the Malpighian tubules take up indigo-carmine so readily, it seems that these organs would also readily excrete poisons contained in the blood. As indigo-carmine does not seem to pass through the walls of the small intestine and those of the rectum, they are either impermeable to this substance or the stain has been so changed that it has lost its original permeability. The same reasoning might also be used for nicotine or any other stomach poison which acts similarly, although according to Cushny (6) iron behaves quite differently when administered to the higher animals. He says:

> Iron injected into the veins of animals is stored up in the liver, spleen and bone marrow, but is taken up from these organs again, and is excreted by the epithelium of the cæcum and colon. When iron is given by the mouth, therefore, it may either pass along the canal and be thrown out in the fæces, or it may be absorbed, make a stay in the liver, be excreted in the large intestine, and again appear in the stools.

He also states that iron has been followed in its course through the tissues by histological methods, but nothing is known about the changes which iron preparations undergo in the stomach and intestine, or the form in which iron is absorbed.

3.—TRACING NICOTINE AS A FUMIGANT TO NERVOUS SYSTEM

While experimenting to determine the physiological effects of nicotine as a fumigant, various insects were fumigated with pure nicotine and a 40 per cent nicotine-sulphate solution. The results indicated that the nicotine fumes were condensed wherever they went. Several of the green peach aphids (*Myzus persicae*) that had been killed by the fumes from the solution of 40 per cent nicotine sulphate were fixed with the mixture consisting of absolute alcohol and phosphomolybdic acid. A careful study of the sections made from these aphids gives the following results:

As already stated, these aphids appeared to be covered with fine spray before they died, indicating that the fumes had changed into tiny drops of liquid. In the sections it is easily seen that the entire integument is covered with minute particles of precipitate. Plate 3, figure B, taken from a molting aphid, well represents the precipitate (*pr*) on the integuments which have been cut obliquely. It is to be noted that the minute precipitated particles lie on the outside of both the old (int_1) and new integuments (*int*), and even between them, but never on the inside of the new integument. Aphids, not fumigated, put into the same fixative occasionally show a little precipitated material on the outside of the integuments, but usually it is easily distinguished from the precipitate

resulting from the union of the fumes of nicotine sulphate and phosphomolybdic acid. It is practically impossible to find an aphid or perhaps any other insect that does not carry at least a little organic matter on the integument. Bees, for instance, when placed into silicotungstic acid or into any other alkaloidal reagent soon become more or less covered with a white precipitate, showing that the hairs are full of organic matter.

It is supposed that some of the nicotine fumes which had passed into the tracheæ had not changed into liquid by the time the insects were fixed, and in order to prove that the fixative precipitates nicotine, whether in a liquid or in a gaseous state, a test tube was filled with the fumes from the nicotine sulphate. Immediately after a small quantity of the fixative was poured into the test tube a yellowish precipitate was thrown down.

Upon examining the sections that had not been stained, a few of them were observed to have a light-tan color and the chitin in places assumed a darker tan color. All the aphids after being fumigated assumed a light-tan color; this color was particularly noticeable when the insects were embedded in white paraffin. The same species of aphids, not fumigated, had a whitish appearance. The light-tan color in most of the sections is caused by minute particles of tan-colored precipitate on the integument, in the tracheæ, and to a limited degree in the tissues, but in a few cases the tan-colored tissues contain no perceptible precipitate. In such instances the fumes must have penetrated the cells and mixed with the protoplasm before the cell constituents were coagulated.

Most of the tracheæ contain more or less tan-colored precipitate, but very little of it lies outside the tracheal walls. Plate 3, figure A, represents a large trachea (*tr*) cut both crosswise and longitudinally near a spiracle, showing the precipitate (*pr*) inside the tracheal walls and some scattered in the fat cells (*fc*) which surround the trachea. Tracheæ may be traced for short distances between the cells of any tissue, but the precipitate is never found further from the tracheæ than that shown in Plate 3, figure A. It is quite probable that some of it which lies in the cells has been dragged there from the tracheæ by the microtome knife.

Baker (2) has well described the respiratory system of the woolly apple aphis (*Eriosoma lanigerum* Hausm.), and he gives two drawings, one representing the dorsal tracheal system and the other the ventral tracheal system. To illustrate how well a gaseous or vaporous form of an insecticide may be distributed to all tissues, these two figures are again given (Pl. 3, fig. D, E). It is to be noted that there are seven pairs of abdominal spiracles and two pairs of thoracic ones and that in the abdomen a short distance from each spiracle the trachea divides into two smaller branches, one of which passes dorsally to help form the dorsal tracheal system (Pl. 3, fig. D) and the other passes ventrally to help form the ventral tracheal system (Pl. 3, fig. E). In the ventral system of the thorax there are two ventral arches, while in the dorsal system of the abdomen there is only one, the dorsal arch (Pl. 3, fig. D, *da*). The anterior ventral arch (Pl. 3,

fig. E, *ava*) in the thorax unites the pair of spiracles in the prothorax and aerates the subesophageal ganglion, whereas the posterior ventral arch (*pva*) connects the pair of spiracles in the metathorax and aerates the large thoracic ganglion. The two ventral arches are of the greatest interest, because they and a few other smaller branches carry nicotine fumes directly to the nervous system, and for this reason it is understandable why the fumes so quickly paralyze aphids.

Plate 3, figure F, is a reproduction of a combination drawing from five consecutive sections through the thorax of an aphid that had been fumigated with a solution of 40 per cent nicotine sulphate, showing the precipitate (*pr*) on the integument (*int*), in the tracheæ (*tr*), and in the subesophageal ganglion (*sg*). The large trachea was cut crosswise near the spiracle, and the branches are drawn in only their approximate positions. It is to be noted that the anterior ventral arch (*ava*) passes over the subesophageal ganglion, but sends one of its branches under and into this ganglion. Another large branch from the main trachea also sends one of its branches to the same ganglion, penetrating its dorsal surface. These sections did not actually show the small tracheæ penetrating this ganglion, but sections from several other aphids did.

Plate 3, figure C, reproduces a combination drawing from six consecutive sections of the same aphid as above described, showing three tracheal branches entering the thoracic ganglion. Attention is to be called to the precipitate (*pr*) in these tracheæ and in the ganglion. Often large granules resembling precipitated particles lie in and near the ganglia. Three groups of them are represented in this figure, two being near the largest trachea and one by the smaller trachea. These fine particles may be either the precipitate resulting from fumes that had passed through the tracheal walls, or that from some other source. There can be no doubt about the large particles of the precipitate, because they are never found in aphids used as controls.

While it is easy to find tracheæ and precipitate in the ganglia, it is quite difficult to find them in the brain. This seems to be due chiefly to the absence of the larger tracheal branches in the brain. Plate 3, figure H, shows a small tracheal branch in the brain cut crosswise, containing three particles of the precipitate, and there are a few more scattered in the adjacent brain. Plate 3, figure G, shows a small tracheal branch running into an optical lobe, containing a few particles of the precipitate.

A critical study of any given tissue would certainly show that it contains as much precipitate as found in the nerve tissue; but no other tissue was thus studied, because all the evidence indicated from the outset that nicotine kills insects by paralysis. One more illustration from the aphid may be used to show that the precipitate may also be found in tracheæ aerating other tissues. Thus, Plate 3, figure I, represents a tracheal branch containing the precipitate running between two ovaries.

Two of the recently emerged house flies that had been fumigated were fixed with the mixture of absolute alcohol and phosphomolybdic acid, and were sectioned. Most of the sections were not cleared well and consequently are not reliable for a study of this kind, but a few of them are fairly reliable. Plate 3, figure M, represents a spiracle and a portion of a trachea taken from the abdomen of a fly. The neck of the spiracle was almost closed with the precipitate (*pr*), while scattered particles of it were seen along the walls of the trachea. Plate 3, figure L, represents two medium-sized tracheæ (*tr*) and a large fat cell (*fc*), taken from another section through the abdomen, showing fine particles of precipitate (*pr*) inside the tracheæ and in the fat cell outside the nucleus.

In conclusion, under this head a few more remarks may be made. A ganglion is composed usually of two more or less round or oblong halves which are securely united to one another. The outer or cortical layer is cellular, while the center of each half never shows definite cell walls or nuclei like those in the cortical layer. There is also usually a difference in coloration between these two portions after being stained, although this difference in the aphids stained with safranin was scarcely noticeable, and the cortical layer was not cellular. This was true not only for those that had been fumigated, but also for the controls that had been fixed and stained the same way. In the illustrations the two portions are distinguished by a difference in stippling. At no time was any anatomical change observed in any insect that could actually be attributed to the effect of nicotine. The failure to see such changes, if they existed, is not significant, because the physical changes effected by the fixation probably mask the smaller physical changes brought about by the nicotine. In the higher animals, however, it has been observed that nicotine causes slight anatomical changes in the cortical layer of the brain.

In cross sections of caterpillars the tracheæ are easily traced into the ganglia. Most of them penetrate the neurilemma and pass between the two halves, where they ramify considerably by sending minute branches through both portions of a ganglion. Occasionally a small tracheal branch may enter a ganglion near or at the base of a nerve. The ramifications of tracheæ inside the ganglia of aphids are not so easily observed, but they seem to exist, although perhaps not so abundantly.

All the preceding histological work has shown that nicotine spray solutions, and even nicotine used as a fumigant, do not penetrate chitin. To determine whether pure nicotine, undiluted, is able to penetrate chitin, larvæ of house flies, of the lesser wax moths, and aphids (*Aphis rumicis*) were submerged in this fluid for 35 minutes. A study of the sections made from these insects showed that the nicotine had passed into the newly-formed chitinous walls of the tracheæ in the larvæ of the house flies and wax moths, but had not passed all the way through them. Plate 3, figures J and K, representing cross sections of small tracheæ, well illus-

trate this point. These tracheal walls certainly were not much harder than the other tissues, because they stained more deeply than did the older chitin. Plate 3, figure O, represents another trachea of the wax moth, showing an older chitinous tracheal wall; the nicotine did not pass into this wall. Plate 3, figure P, represents a small portion of the integument (*int*) of an aphid, showing that the pure nicotine did not pass into the chitin. Plate 3, figures N and Q, illustrates how well Carnoy's fluid penetrates hard chitin. The black dots in the illustrations (*pr*) are crystals of mercuric chlorid that have remained after the fixative was removed. Plate 3, figure N, represents a trachea from a wax-moth larva, and Plate 3, figure Q, a portion of the integument (*int*) and fat cells (*fc*) from an aphid. Attention is called to the mechanical or physical changes brought about in the fat cells by the fixation. Live fat cells never have a netlike appearance, but appear more or less granular, and usually contain many globules. Sections from other insects that had been fixed in Carnoy's fluid showed better than does figure Q the ability of this fluid to penetrate the integument. In a few cases the crystals lie in rows penetrating the integument, indicating that the fluid had passed through the chitin in streams.

If the most important results recorded under this large heading are briefly summarized, the following conclusions may be drawn: (1) Nicotine spray solutions neither enter the spiracles nor pass through the integuments of insects; (2) nicotine as a stomach poison seems to be distributed to all the tissues, including the nervous system; (3) nearly all the nicotine fumes that strike the integuments and pass into the tracheæ are immediately condensed, so that in regard to nicotine as a fumigant the integuments and tracheal walls are more or less covered with fine spray; (4) this fine spray is well distributed through the many small tracheal branches to all the tissues, where some of it passes into the cells; (5) the nervous system receives its quota of the fine spray and vapors from the spray, which immediately paralyzes the nerve cells; (6) the statement just preceding explains how odoriferous particles and vapors from nicotine spray solutions kill insects by paralysis.

HISTORICAL REVIEW

After making a few remarks concerning the chemistry and properties of nicotine, a brief review pertaining to the pharmacological effects of nicotine on various classes of animals, and a few other observations by the writer will be given in order that these results may be compared with those obtained on the insects discussed in the preceding pages.

(1) GENERAL REMARKS ABOUT NICOTINE

Nicotine ($C_{10}H_{14}N_2$) was conclusively prepared synthetically by Pictet and Rotschy (19) in 1904. This investigation concluded a long series of works pertaining to the structure of this deadly poisonous alkaloid. These authors showed that it is a pyridin-methyl-pyrrolidin.

Blyth (3, p. 271–272) says that nicotine—

When pure, is an oily, colorless fluid, of 1.0111 specific gravity at 15°. It evaporates under 100° in white clouds, and boils at 240°, at which temperature it partly distils over unchanged, and is partly decomposed—a strong resinous product remaining. . . . It has a strong alkaline reaction . . . and a sharp caustic taste. It absorbs water exposed to the air, and dissolves in water in all proportions, partly separating from such solution on the addition of a caustic alkali.

The aqueous solution acts in many respects like ammonia, saturating acids fully; and by the action of light pure nicotine soon becomes yellow, then brown and thick, in which state it leaves, on evaporation, a brown resinous substance.

(2) PHARMACOLOGICAL EFFECTS OF NICOTINE ON VARIOUS CLASSES OF ANIMALS

Greenwood (9) experimented on certain protozoa, cœlenterates, the earthworm, certain echinoderms, crustaceans, and on certain mollusks by using nicotine. He found that the toxic effect of this alkaloid on any organism is determined mainly by the degree of development of the nervous system. Thus, for the protozoa that he used it can not be regarded as exciting or paralyzing, but is rather inimical to continued healthy life. He states (p. 604) that—

As soon as any structural complexity is reached the action of nicotin is discriminating, and discriminating in such a fashion that the nervous actions which are the expression of automatism—which imply coordination of impulse—are stopped first. This is seen dimly in Hydra, and it is more pronounced among the medusae, where spontaneity, irradiation of impulse and direct motor activity are affected successively.

He asserts that relative to the higher invertebrates the paralyzing action of nicotine is preceded by a phase of stimulation; and as the positively exciting action becomes noticeable, nicotine becomes more and more a medium in which life is impossible. He found that animals closely allied structurally may also often behave quite differently toward nicotine.

The present writer carried on one preliminary experiment to ascertain the action of nicotine on the lower invertebrates. A piece of scum containing many paramecia and nematodes was placed on a slide under a cover glass. A drop of pure nicotine was then placed at the edge of the cover glass and the following results were observed. The nicotine gradually passed under the cover glass by mixing with the water, and as quickly as it came in contact with the nematodes they began to squirm vigorously, while the paramecia apparently were not affected. A little later the nematodes formed themselves into spirals and lay apparently paresized; then suddenly the spirals unfolded. This kind of behavior continued until the nematodes were no longer able to move. By this time it was observed that the nicotine had passed into their bodies, and later the tough cuticles were constricted and contained many

deep grooves. The paramecia were still alive when the nematodes became lifeless, but they finally died slowly and gradually and at no time showed any reaction which could be attributed to a stimulation.

There are many papers dealing with the economic importance of nicotine as an insecticide, but they contain nothing about the pharmacological effects of nicotine and little about its physiological effects, except that it is effective.

So far as known to the writer, only two authors have anything to say about the pharmacological effects of nicotine on insects.

Del Guercio (10) sprayed silkworms with various dilutions of nicotine and determined that this insecticide within a short time brings about convulsive movements in the caterpillars, causing them to fall from the plants and resulting in death in most cases. He thinks that nicotine spray solutions affect insects by means of the vapors from the nicotine poisoning them and that these vapors even in minute quantities cause irritation and convulsive movements which result in death by total paralysis. He made no histological study to ascertain what tissue is vitally affected, and his view is based solely on the behavior of the caterpillars treated.

Shafer (20) ascertained that insects subjected to the vapors of nicotine and other contact insecticides first pass through a stage of excitement, then through a stage of depression in which the coordination of movements is uncertain, and finally through a stage in which there is total loss of movement and sensibility. The last stage was followed more or less rapidly by death. During the first stage the action of the heart was increased and was irregular, then it became depressed, but the heart action was one of the last visible signs of life to disappear. Secretions were also observed to issue from the mouths. The value of the respiratory ratio arose, showing that these vapors depress the activity of oxygen absorption more than they do the ability of carbon-dioxid excretion. Shafer found that the insects used continued to give off carbon dioxid when no oxygen was present to be taken up. Loeb (16) cites similar experiments in which muscles deprived of oxygen continued to give off carbon dioxid.

Before discussing the pharmacological effects of nicotine on the vertebrates, the physiological classification of this alkaloid as defined by toxicologists may be given. Blyth (3, p. 269–279) places nicotine in that class of poisons affecting the nervous system which causes convulsive movements and complex nervous phenomena. Kobert (12) places nicotine in that class of poisons affecting the cerebrospinal system which is able to kill without producing coarse anatomical changes. Brundage (4) classifies nicotine as a neurotic which depresses the cerebrospinal system.

Blyth (3) says that small fish die within a few minutes from a milligram of nicotine. They are first stimulated, then become less active, and are rapidly paralyzed.

The successive stages of nicotine poisoning in the frog are briefly summarized by Langley and Dickinson (14) as follows: (1) Stage of excitation; (2) stage of spasms; (3) stage of quiescence; (4) stage of flaccidity; (5) stage of paralysis of the central nervous system; (6) stage of paralysis of the motor-nerve endings.

Blyth (3, p. 273) says, "Birds also show tetanic convulsions, followed by paralysis and speedy death," and Sollmann (23, p. 262) asserts that—

> Nicotine is one of the most fatal and rapid of poisons; the vapor arising from a glass rod moistened with it and brought near the beak of a small bird causes it to drop dead at once, and two drops placed on the gums of a dog may cause a similar result.

According to Langley and Dickinson (14), the symptoms of nicotine poisoning in rabbits, cats, and dogs are in a general way similar, and may be briefly described as follows: There is a preliminary excitement; clonic spasms; twitchings of the muscles in various parts of the body; stimulation of the central nervous system; paralysis of the motor-nerve endings in the skeletal muscles; quickening and deepening of the respiration, followed by slowing and cessation; dilation of the pupils; paralysis of the cervical sympathetic system; rise and fall of the blood pressure; rise of temperature; constriction of intestines, followed by dilation, and slight vomiting in cats and dogs. If the doses are sufficiently large, the cerebrospinal system is totally paralyzed.

According to Blyth (3), the symptoms witnessed in mammals poisoned by nicotine are quite similar. With large doses, there is a cry, one or two shuddering convulsions, and death; with smaller doses, there is trembling of the limbs, excretion of feces and urine, stupor, a staggering gait, and then the animal falls on one side. One or two drops of pure nicotine may kill a rabbit, cat, or dog within five minutes. Vas (24) found that the substance resulting after washing tobacco smoke affects the health of rabbits; they lose weight, the number of blood corpuscles is decreased, and the hemoglobin of the blood is diminished. According to Blyth, nicotine also affects horses similarly to the smaller domestic animals.

Blyth says that Dragendorff ascertained that nicotine is absorbed into the blood and is excreted unchanged, in part by the kidneys and in part by the salivary glands.

Krocker (13) was among the first investigators to determine the pharmacological effects of nicotine on man. He found that it paralyzes the nervous system and that death is caused by the rapid benumbing and paralysis of the respiratory center, but not from heart paralysis, although nicotine powerfully influences the action of the heart.

Holland (11) states that two or three drops of the alkaloid is fatally poisonous to man when taken into the stomach, and that death is caused by heart failure. In this latter statement other authorities do not agree with him, for they say that death is due to asphyxia, on account of the paralysis of the respiratory center. In the lower vertebrates the heart still beats some time after life is extinct.

Sollmann (23) summarizes the symptoms of nicotine poisoning on man as follows: The whole cerebrospinal axis is first stimulated, then depressed from above downward; symptoms from large doses resemble those of asphyxia or hydrocyanic acid. Action on the medullary centers is marked and violent; respiration is at first increased, then markedly depressed; paralysis of the respiratory center is the cause of death. Action on the spinal cord consists in strong stimulation of the motor cells, producing convulsions, passage of feces and urine. Nicotine acts on unstriped muscles, paralyzing the ganglia after a brief stimulation. There is nausea and vomiting, violent peristalsis, and even tetanic contraction of the intestine and diarrhea. The respiration, heart strength, and blood pressure are increased; the heart rate is decreased. A strong nicotine solution applied directly paralyzes the nerve fibers. "Free nicotine is caustic on account of its alkalinity." The fatal dose for man is about 60 mgm.; one cigar contains enough nicotine to kill two persons, if it were directly injected into the circulation. "It acts with a swiftness only equaled by hydrocyanic acid."

Cushny (6, p. 304–314) asserts that poisonous doses of nicotine administered to man or other mammals cause a hot, burning sensation in the mouth which spreads down the esophagus to the stomach and is followed by salivation, nausea, vomiting, and sometimes purging. Mental confusion, muscular weakness, giddiness, and restlessness are followed by loss of coördination and partial or complete unconsciousness. Clonic convulsions set in later and eventually a tetanic spasm closes the scene by arresting the respiration.

Autenrieth (1, p. 87) says that nicotine is absorbed from the tongue, eye, and rectum within a few seconds, but from the stomach somewhat more slowly. Its absorption is also possible from the outer skin, and it is eliminated through the lungs and kidneys.

In concentrated form nicotine is a local irritant, though owing to the rapidity of its toxic action, it does not behave like a true corrosive nor does it cause inflammation of the mucous lining of the stomach after a lethal dose.

(3) PHYSICAL AND CHEMICAL EFFECTS ON THE CELLS

In the preceding discussion it has been pointed out that nicotine paralyzes the respiratory center in the brain of vertebrates, causing death by asphyxiation. This implies that, while the nervous system is benumbed and rendered inactive, the lungs are prevented from functioning, and consequently the cells in the tissue die for want of oxygen. Since the invertebrates are differently organized, particularly in regard to their respiratory system, an investigation will be made as to the period insects can live without free oxygen.

Walling (25) states that grasshoppers confined in pure carbon dioxid for 15 hours recover, and Shafer (20) determined that beetles (*Passalus*

cornutus Fab.) confined for 24 hours in pure hydrogen completely recover when placed in fresh air. These experiments indicate that nicotine does not kill insects merely on account of the paralysis of the respiratory centers, because the tracheæ and tissues of an insect contain enough oxygen to keep the cells alive for several hours. Since the cells of insects are constantly surrounded by air containing oxygen, an investigation will be made as to whether or not nicotine interferes with oxidation in the cells and whether it kills physically or chemically.

Greenwood (9) observed that when simple animals die from the effects of nicotine, death is often associated with injury to the cell contents so that they tend to disintegrate. This is shown in the protozoan, *Actinosphaerium* sp., and in the coelenterates, *Hydra* spp. and *Medusa* spp.

Budgett (5) treated infusorians with a number of poisons, including nicotine, and found that these protozoa become strongly vacuolated and finally the membranes burst, allowing the protoplasm to flow out into the water. The same structural changes occurred when he deprived them of oxygen. He says (p. 214): "This indicates that either these poisons prevent oxidation or that lack of oxygen produces toxic substances." He also believes that these poisons not only reduce the normal resistance to the entrance of water but lead to the taking up of water, probably by hastening the molecular breakdown and so increasing the osmotic pressure within the cell.

Loeb (16) and others have experimented extensively with amebæ and paramecia by depriving them of oxygen. They always observed the same structural changes as already cited from Budgett. Loeb also performed many experiments by depriving the eggs of a certain fish of oxygen. He exposed the eggs to a current of hydrogen and observed—

> The liquefaction of the cell walls and the formation of droplets began when the egg was in the 8-cell stage (Fig. 2). These droplets fuse into larger drops and finally nothing but these drops indicates the existence of the germinal disk.

The present writer placed living fat cells and œnocytes of the honeybee on a slide in water under a cover glass. These cells live in tap water for some time before any changes in their appearance can be observed; but when a drop of pure nicotine is placed at the edge of the cover glass, changes in their general appearance take place soon afterwards. The globules in the cells sometimes dance about, resembling the Brownian movement. The globules in the fat cells usually soon lose their rotundity, become massed together, and form a coarse, granular structure. The refractive bodies in the œnocytes soon disappear, and then these cells become opaque. After considerable time the cell walls of the fat cells and œnocytes burst, and the cell contents disintegrate.

In the preceding pages it is shown that either lack of oxygen or the presence of nicotine around simple animals brings about structural changes resulting in death. A comparison, although a rough one, might

also be made between the fish eggs used by Loeb and the fat cells and œnocytes employed by the present writer. All these facts seem to indicate that either lack of oxygen or the presence of nicotine around the cells kills physically rather than chemically. The following paragraphs will considerably strengthen this statement.

It is well known that different fluids have different osmotic pressures, and this is also true for the blood of different animals. In order that tissues removed from various animals might be kept alive for some time, Lewis (15) has shown that they must be placed in fluids having different osmotic pressures. Endeavoring to make a fluid having an osmotic pressure equal to that of the blood in a grasshopper, Lewis used sea water, distilled water, grasshopper bouillon, sodium bicarbonate, and dextrose. The effect of osmotic pressure on cells is best illustrated by using red corpuscles. According to Cushny (6, p. 304–314), water passes into these cells readily and when placed into distilled water they swell up and burst, but when placed into an aqueous solution of sodium chlorid having an osmotic pressure greater than that of their contents, they shrink because the contained salts are unable to retain water against a higher concentration outside. A change brought about in the osmotic pressure of the blood might be a probable explanation of the death of the honeybees recently fed various salts by the present writer (18).

There are several theories regarding the manner in which drugs and powerful poisons affect the cells. Cushny has briefly summarized them about as follows: (1) Some drugs enter into definite chemical combinations with the constituent protoplasm; (2) some drugs act on the cells by changing the relation of the cell constituents in which they are dissolved; (3) some drugs alter the surface tension of the cells in relation to the surrounding fluids; (4) a few powerful drugs may act by altering the surfaces of the cells without penetrating into the interior; (5) many drugs may change the intracellular membranes; and (6) other drugs may reduce the permeability of the cellular membranes by altering their electric charges. Cushny says (6):

> From the present confusion the only legitimate conclusion seems to be that the activity of drugs depends on a large variety of factors and that pharmacological action can not be brought under any one law, either chemical or physical.

Shafer (21) has added another view which should be classified with the chemical ones, for it deals with the enzym-like cell constituents which accomplish oxidation. He thinks that contact insecticides, nicotine included, deleteriously affect the activities of the reductases, catalases, and oxidases in an unequal degree, thereby disturbing the natural or normal balance of the activities of these enzym-like factors.

SUMMARY

(1) Nicotine spray solutions do not pass into the tracheæ, nor do they penetrate the integuments of insects.

(2) The fumes from nicotine used as a fumigant, the vapors from nicotine spray solutions, and the odoriferous particles from evaporated nicotine spray solutions or from powdered tobacco pass into the tracheæ and are widely distributed to all the tissues.

(3) Regardless of how it is applied, whenever nicotine kills insects, as well as all other animals, it kills by paralysis, which in insects travels along the ventral nerve cord from the abdomen to the brain.

(4) The writer does not know just how nicotine paralyzes the nervous system, but he does know that it prevents the nerve cells from functioning, and that in regard to the simplest animals its presence around the cells causes the same structural changes resulting in death as observed when other animals of the same kind are deprived of oxygen. In such cases it seems to kill physically rather than chemically, but the evidence presented does not conclusively prove this view. In the higher animals it may kill by interfering with oxidation in the cells; whether this is accomplished physically or chemically the writer does not know, but concluding from the properties of nicotine he is inclined to attribute more to its physical effects than to its chemical effects.

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PLATE 1

Fig. A.—Portion of the large longitudinal trachea of the house fly cut crosswise obliquely, showing the carmine acid "precipitate" *pr*. ×190. The fly had been submerged for one hour in a pure nicotine solution (1 : 500) colored with carmine acid.

Fig. B.—Combination drawing from two consecutive sections of a green peach aphis, showing the indigo-carmine "precipitate" *pr* in a trachea *tr*. ×500. The aphis had been submerged for 45 minutes in a pure nicotine solution (1 : 500) colored with indigo-carmine.

Fig. C.—Cross section of a large longitudinal trachea of larva of lesser wax moth, showing the indigo-carmine "precipitate" *pr* adhering to the tracheal wall *trw*. ×190. The larva had been submerged for 30 minutes in a pure nicotine solution (1:500) colored with indigo-carmine.

Fig. D.—Longitudinal section of one of the smallest tracheæ of the same larva as in figure C; same treatment and same enlargement.

Fig. E.—Longitudinal section of a large trachea and one of its branches of a coccid, showing the "precipitate" *pr* resulting from the union of pure nicotine and phosphomolybdic acid. ×500. The coccid had been submerged for 30 minutes in a pure nicotine solution (1 : 100).

Fig. F.—Portion of a cross section of an aphid (*Aphis rumicis*), showing the indigo-carmine "precipitate" *pr* in a spiracle *sp*. ×320. The aphid had been heavily sprayed with a pure nicotine solution (1 : 500) colored with indigo-carmine.

Fig. G.—Portion of a cross section of the same aphid as in figure F, showing no precipitate in the trachea *tr*, but much on the outside of the integument *int*. × 320.

Fig. H–O.—Longitudinal sections of spiracles *sp* with connecting tracheæ *tr*, showing how it is practically impossible for aqueous spray solutions to enter spiracles, owing to hairs *hr*, a closing plate *p*, and a peculiar arrangement of rims *r* at mouths of spiracles.

Fig. H.—Spiracle of a coccid (*Orthezia insignis*). × 500.

Fig. I.—Spiracle of a caterpillar of *Atteva aurea*. × 190.

Fig. J.—Spiracle of a larva of lesser wax moth (*Achroia grisella*). × 190.

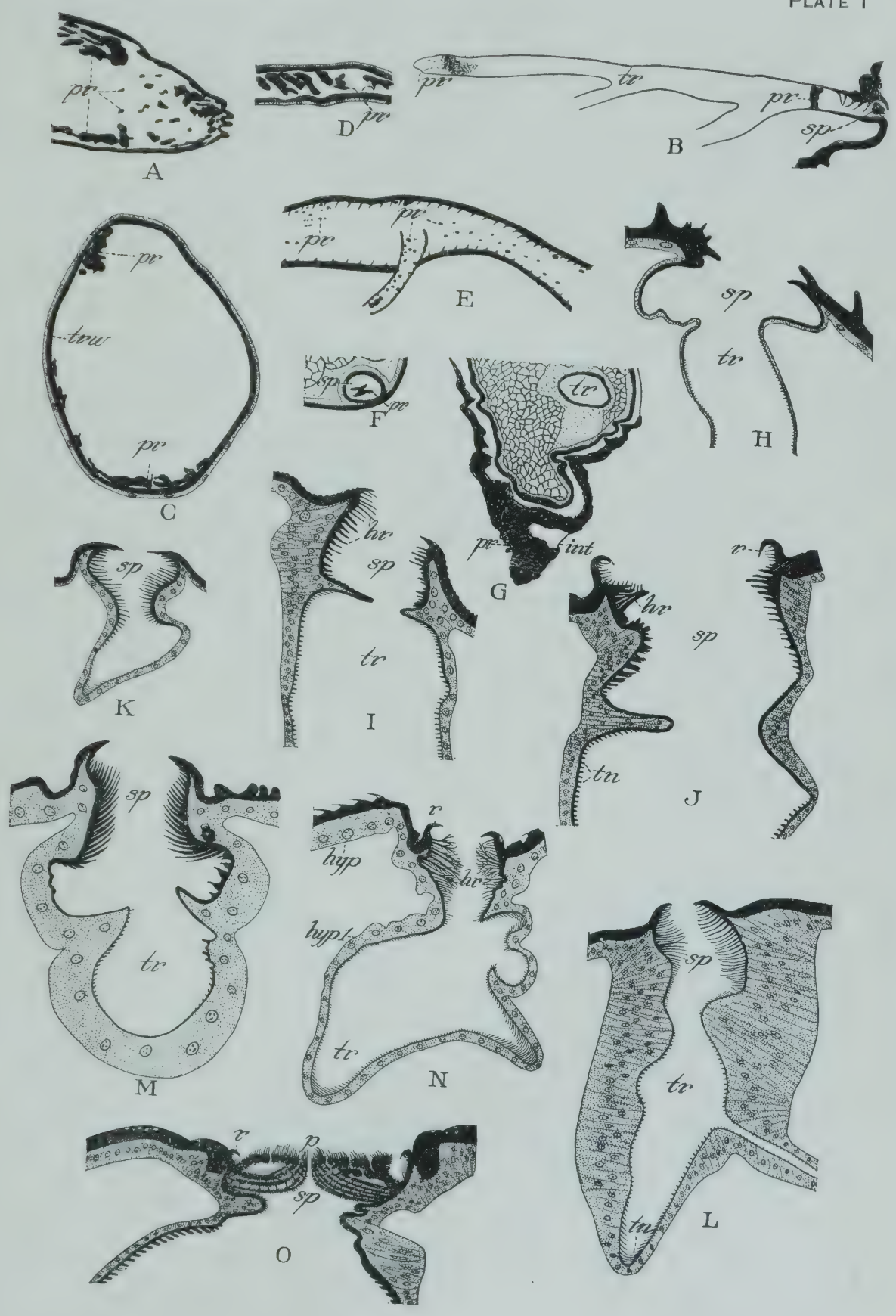
Fig. K.—Spiracle of a caterpillar of *Datana* sp. × 500.

Fig. L.—Spiracle of a caterpillar of a catalpa sphinx (*Ceratomia catalpae*.) × 500.

Fig. M.—Spiracle of a larva of a Colorado potato beetle (*Leptinotarsa decemlineata*). × 500.

Fig. N.—Spiracle of fall webworm (caterpillar of *Hyphantria cunea*). × 320.

Fig. O.—Spiracle of the tomato worm (larva of *Phlegethontius sexta*), showing the closing plate *p*. × 50.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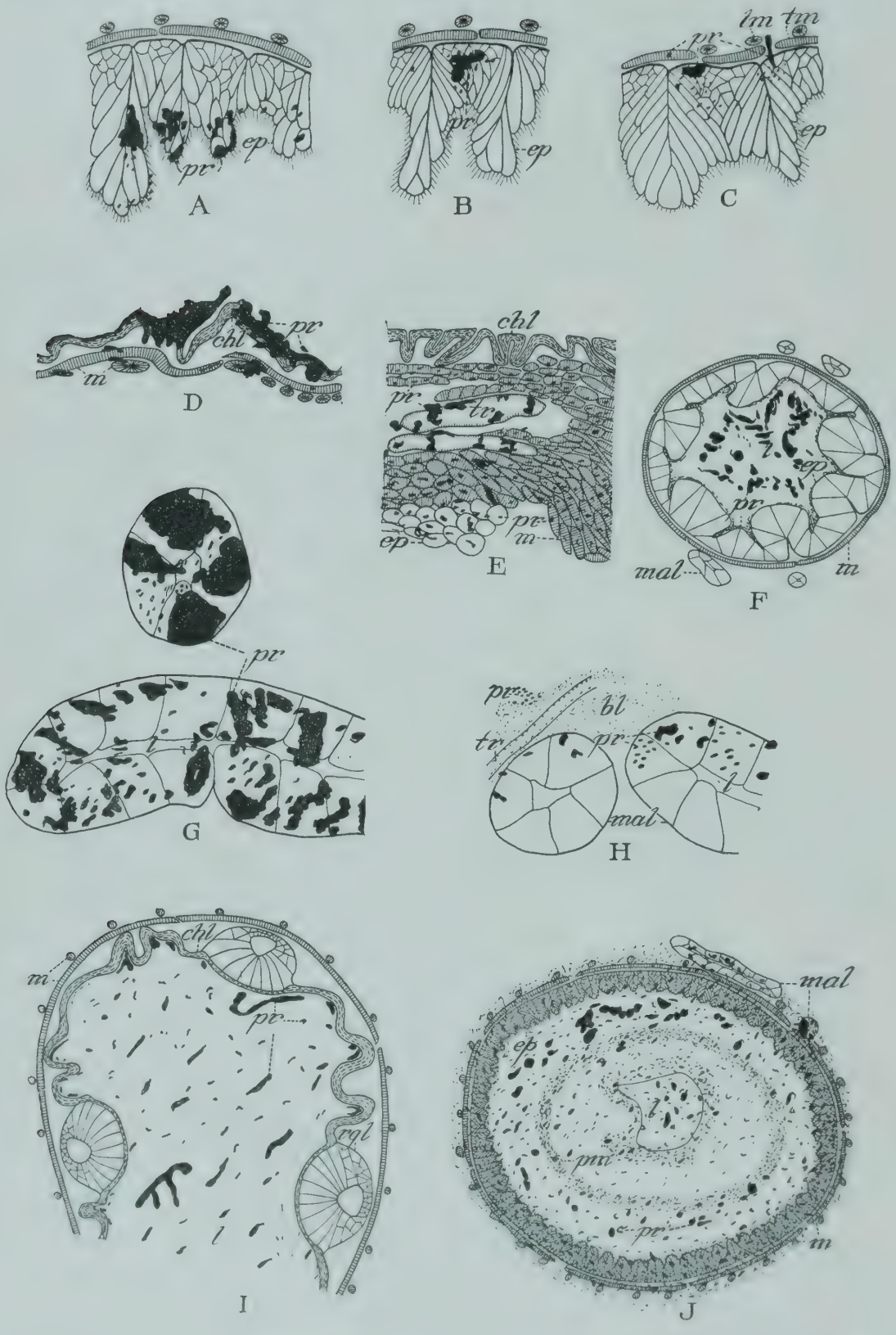
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PLATE 2

Fig. A to J.—Cross sections of portions of the alimentary canals and Malpighian tubules of worker honeybees, showing "precipitated" indigo-carmine that had been fed with pure nicotine and honey to bees three days before they were fixed in absolute alcohol. Owing to poor fixation, most of cells are drawn diagrammatically.

Fig. A.—Portion of the wall of the ventriculus, showing the "precipitate" *pr* in inner ends of the epithelial cells *ep*. ×320.

Fig. B.—Portion of the wall of the ventriculus, showing the "precipitate" *pr* in the middle of the epithelial cells *ep*. ×320.

Fig. C.—Portion of the wall of the ventriculus, showing the "precipitate" in the outer ends of the epithelial cells *ep* and in the transverse muscle layer *tm*. ×320.

Fig. D.—Portion of the wall of the honey stomach joining the proventriculus, showing the "precipitate" *pr* in the chitinous *chl* and muscular *m* layers. ×320.

Fig. E.—Portion of the wall of the anterior part of the valve of the proventriculus, showing the "precipitate" *pr* in muscles *m*, tracheæ *tr*, and epithelial cells *ep*. ×320.

Fig. F.—Section through the small intestine, showing the "precipitate" *pr* in the center of the lumen *l* and lining epithelium *ep*, but none in the walls of this organ nor in the Malpighian tubules *mal* by it. ×50.

Fig. G.—Section through two Malpighian tubules *mal* against the ventriculus, showing the "precipitate" *pr* in their cells and lumens *l*. ×320.

Fig. H.—Section through two Malpighian tubules *mal* near the ventriculus, tracheal branch *tr* and blood *bl*, showing the "precipitate" *pr* in these tissues. ×320.

Fig. I.—Section of one-third of the rectum in a compressed condition, showing the "precipitate" *pr* in the lumen *l*, but none in the chitinous layer *chl*, nor in the rectal glands *rgl*, nor in the muscular *m* layer. ×50.

Fig. J.—Section through the middle of the ventriculus, showing the distribution of the "precipitate" *pr* in the lumen *l*, between the peritrophic membranes *pm*, in the epithelial *ep* and muscular *m* layers of the ventriculus and in the Malpighian tubules *mal*. ×50. The walls of the ventriculus were drawn diagrammatically.

PLATE 3

Fig. A to I, L, M.—Drawings and diagrams representing the distribution of precipitate resulting from the fumes of 40 per cent nicotine sulphate and phosphomolybdic acid. Figures A, B, F to I are from the same green peach aphis, and figures L and M are from the same house fly that had been fumigated. × 320. Figures D and E are diagrams, after Baker (2), representing the respiratory system of an aphid, *Eriosoma lanigerum*.

Fig. A.—Transverse-longitudinal section of a trachea of an aphid, showing the precipitate *pr* inside the trachea and in fat cells *fc* near by.

Fig. B.—Cross section of a portion of an aphid just molting, showing the precipitate *pr* on the outer surfaces of the old int_1 and the new integuments *int* and between them, but none in the fat cells *fc*.

Fig. C.—Combination drawing from six consecutive sections through thoracic ganglion of an aphid, showing the precipitate *pr* in three tracheal branches *tr* in the cortical layer *cl* and in the inner layer *il* of a ganglion.

Fig. D.—Diagram of the dorsal tracheal system of an aphid, showing the dorsal trunk *dt* and dorsal arch *da*.

Fig. E.—Diagram of the ventral tracheal system of an aphid, showing the anterior ventral arch *ava*, posterior ventral arch *pva*, and the ventral trunk *vt*.

Fig. F.—Combination drawing from five consecutive sections through the thorax of an aphid, showing the precipitate *pr* on the outer surface of the integument *int*, in the tracheæ *tr* and in the subesophageal ganglion *sg*.

Fig. G.—Portion of cross section of an optic lobe of an aphid, showing the precipitate *pr* inside and outside a tracheal branch *tr*.

Fig. H.—Cross section of the brain *br* and optic lobes *opl* of an aphid, showing the precipitate *pr* in the tracheal branch *tr* and in the cortical layer of the brain.

Fig. I.—Cross section of two ovaries *ov* of an aphid, showing the tracheal branch *tr* containing precipitate passing between them.

Fig. L.—Cross section of two tracheæ *tr* and a fat cell *fc* of a house fly, showing the precipitate *pr* in the tracheæ and in the fat cell outside its nucleus.

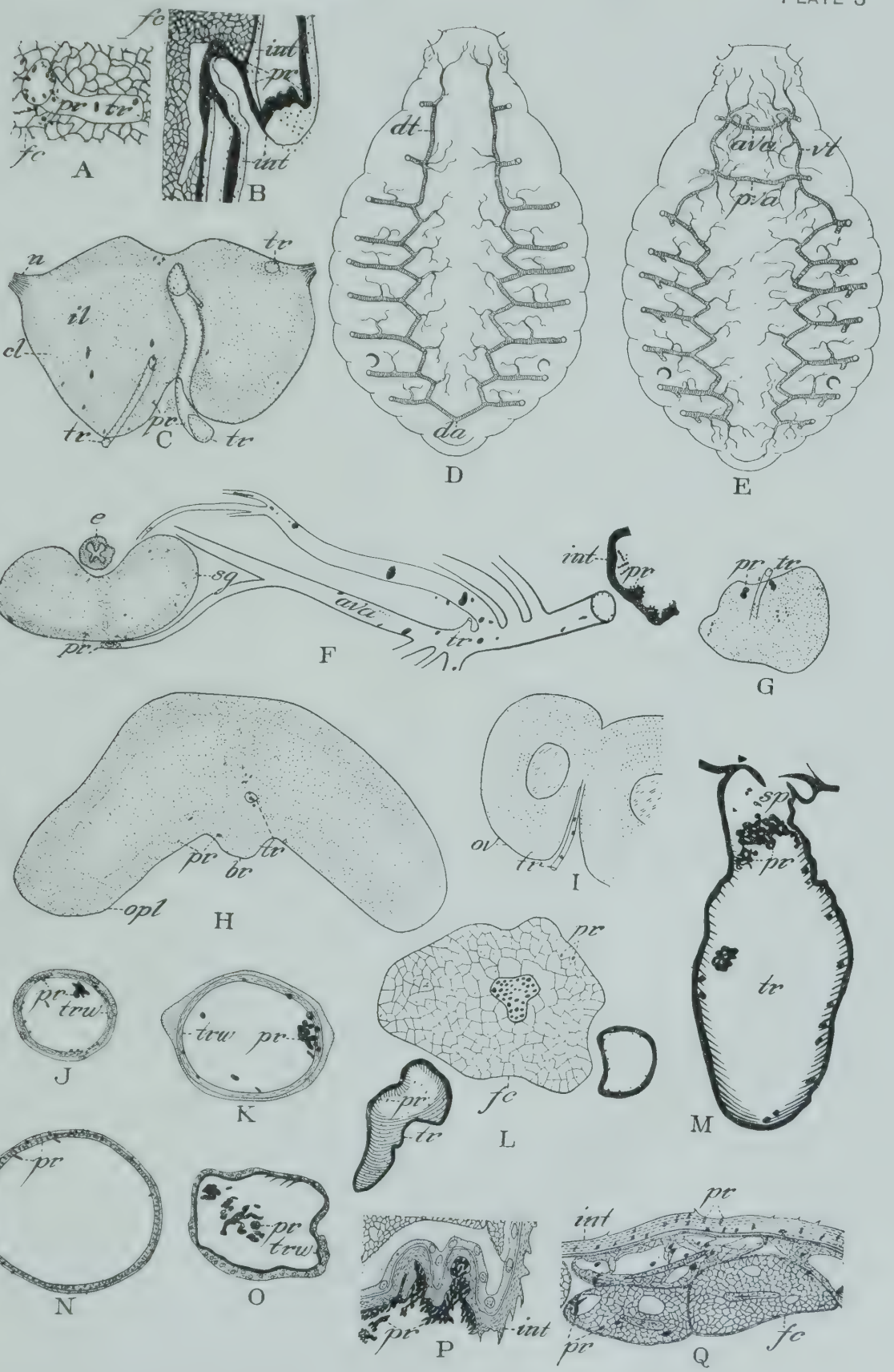
Fig. M.—Longitudinal section through a spiracle *sp* and its connecting trachea *tr* of a house fly, showing the precipitate *pr* in the neck of the spiracle and along the tracheal wall.

Fig. J and K.—Cross sections of the small tracheæ, showing the precipitate *pr* in newly formed tracheal walls *trw* resulting from the union of pure nicotine and phosphomolybdic acid. These and other insects had been submerged in pure nicotine for 35 minutes. × 190. Figure J is from a house-fly larva and figure K from a lesser wax-moth larva.

Fig. N and Q.—Cross sections, showing how well Carnoy's fluid passes through hard chitin, as indicated by remaining crystals *pr* of mercuric chlorid. Figure N is a trachea from a lesser wax-moth larva. × 190. Figure Q shows a portion of the integument *int* and the fat cells *fc* of an aphid, also showing the physical change in the fat cells caused by a fixative. × 500.

Fig. O.—Cross section of a medium-sized trachea of a lesser wax-moth larva, showing that pure nicotine did not pass into an older tracheal wall *trw* under the same conditions as stated for figure K. × 190.

Fig. P.—Cross section of portion of the integument *int* of an aphid (*Aphis rumicis*), showing that pure nicotine did not pass into chitin under same conditions as stated for figures J, K, and O. × 500.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ACIDITY AND ADSORPTION IN SOILS AS MEASURED BY THE HYDROGEN ELECTRODE

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INTRODUCTION

The problem of soil reaction has come to occupy an increasingly important position in the realm of soil-fertility studies. This is evident from the numerous papers recently appearing upon the subject of soil acidity. The great diversity of opinion concerning the nature and methods of measuring soil acidity has served thus far only to confuse the matter. By adopting modern methods capable of measuring specifically the hydrogen ion,[2] data have been obtained by the writers which seem to offer a clearer conception of the question.

Before considering the question of soil acidity, it seems desirable to recall the fundamental significance of the terms "neutrality," "acidity," and "alkalinity." The dissociation of pure water produces H ions and OH ions in equal concentration, denoting neutrality. The product of the concentrations of these ions in any solution is a constant, approximately 1×10^{-14}. When the H ions are present in a concentration greater than the OH ions—that is, in a concentration greater than 1×10^{-7}, then the resulting solution is acid. Conversely, the presence of OH ions in greater concentration than 1×10^{-7} gives an alkaline solution. The term "acidity" is often construed to mean the total quantity of H ions which may be produced when the equilibrium is continually shifted by the introduction of OH-ions. This total acidity is referred to as potential acidity, while the H-ion concentration at any given moment determines the intensity of acidity, to use Gillespie's (13)[3] expression. In all probability excessive concentrations of H or OH ions in soil solutions exercise pronounced effects on plant growth and on the activities of soil bacteria.

Potential acidity or alkalinity may be due to undissolved substances or to soluble compounds only partly hydrolyzed or dissociated. An obvious illustration of this is afforded by the titration of a suspension of calcium carbonate in water. So long as solid calcium carbonate remains,

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[1] From the Divisions of Soil Chemistry and Bacteriology and Agricultural Chemistry in equal cooperation. Acknowledgment is made to Mr. C. L. A. Schmidt for valuable suggestion during the progress of this work.

[2] To be more exact, according to recent physical-chemical views, it is the activity of the H ion, rather than the concentration, which is measured here. However, for the present purpose this distinction seems unimportant.

[3] Reference is made by number to "Literature cited," p. 143–145.

there will be a definite concentration of H and OH ions, while with the addition of acid the reaction of $H^+ + OH^- = H_2O$ occurs and equilibrium is restored by the solution of more calcium carbonate.

Soil acidity should not be set apart and considered as a phenomenon unrelated to the ordinary concepts of acidity. The use of such terms as "apparent acidity," "real acidity," "adsorption acidity" has led to a confusion of ideas, and, hence, should be discarded. With this in view, it seems desirable to emphasize the generally neglected point of view that the equilibria resulting in the production of H ions are in their essential nature the same for soil solutions as for other simpler chemical systems.

The methods now in vogue for estimating the lime requirement (soil acidity) have often proved to be unsatisfactory and in any case do not measure the intensity of acidity—that is, the H-ion concentration. In order to secure reliable data upon these points, the method of determining the H-ion concentration by means of a hydrogen electrode was used. Some preliminary experiments led to the adoption of a modification of Hildebrand's (17) apparatus, which will be described in detail later. Few similar measurements with soils have been previously recorded. The work of Saidel (29) was limited to a few alkaline soil extracts whose reaction had been changed by boiling. For reasons discussed later, data obtained on soil extracts are not convincing. Fischer (12) also made a few hydrogen-ion determinations with soil. In a more noteworthy investigation, Gillespie (13) has measured the H-ion concentration of 22 soils in water suspensions. The investigations reported in this paper were begun before the appearance of Gillespie's article. The present authors have extended the work into various other phases, such as titration with bases as a means of adjusting the soil reaction and of studying the general phenomena grouped under the term "adsorption." Moreover, various factors affecting the H-ion concentration of soil suspensions were also considered.

VARIOUS POINTS INVESTIGATED

In the course of these investigations several related questions were studied, in addition to the determinations of the H-ion concentration in various soil suspensions and in soil extracts. Experiments were undertaken in order to obtain further evidence regarding the influence of varying proportions of soil to water, grinding the soil, heating it at various temperatures, and of the addition of salts on the H-ion concentration. Consideration has also been given to the relation of HCO_3^-, CO_3^-, and CO_2 to soil reaction as measured by the electrometric method. Experimental data have likewise been secured with respect to the lime requirement of soils and the so-called "adsorption of bases." Finally, the apparatus and methods of procedure are described.

EXPERIMENTAL WORK

MATERIALS USED

Before taking up the detailed discussion of the data it appears desirable to describe briefly the materials used. Table I contains a list and description of all the soils referred to in this paper.

TABLE I.—*Description of soils used in experimentation*

Laboratory No.	Source of soil.	General type.	Remarks.
1	California	Silty clay loam	
2	do	do	
3	do	do	
4	do	do	
5	do	do	
6	do	do	
7	do	Fine sandy loam	
8	do	do	
9	do	do	
10	do	do	
11	do	do	Alkali soil.
12	do	Sandy loam	
13	do	Gravelly loam	
14	do	Clay adobe	
15	do	Fine sandy loam	Very infertile.
16	Wisconsin	Silty clay loam	
17	do	Sandy loam	
18	Pennsylvania	Silty loam	
19	California	Fine sandy loam	
20	Louisiana	Silty loam	
21	California	Peat	
22	Wisconsin	do	
23	California	Sandy loam	Alkali soil.
24	do	Fine sandy loam	Do.

HYDROGEN-ION CONCENTRATION OF SOIL SUSPENSIONS

Table II gives the amounts of soil and water used for making the suspensions. The soils were air-dried and passed through a 1-mm. sieve. The H-ion concentration is expressed in the customary units of gram molecules of H ion per liter.

In accordance with our preliminary statements, Table II gives evidence that soils may give rise to acid solutions—that is, solutions containing a preponderance of H ions over OH ions. This conception is in agreement with the conclusions which Truog (33) drew from his zinc-sulphid method. The work of Gillespie (13), paralleled by that presented in Table II, conclusively proves that there may be an excess of H ions in the solution bathing the soil particles.

Out of 22 soils examined by Gillespie (13), 17 were found to yield acid solutions. The writers experimented with 9 acid soils. The soils were of widely different types and origin. These facts do not support

the views of Cameron (5) concerning soil acidity, that it is generally due to the selective absorption of bases. They also are in opposition to the recent conclusions of Rice (26) and Harris (15), that water-soluble acids are not characteristic of acid soils.

TABLE II.—*Hydrogen-ion concentrations of suspensions of unground soil*

Soil No.	Quantity of soil.	Water.	Readings on voltmeter.	H-ion concentration (gram-molecules per liter).
	Gm.	*C. c.*		
1	2.0	30	0.763	0.4×10^{-7}
2	2.0	30	.753	$.6\times10^{-7}$
3	2.0	30	.759	$.5\times10^{-7}$
4	2.0	30	.763	$.4\times10^{-7}$
5	2.0	30	.759	$.5\times10^{-7}$
6	2.0	30	.753	$.6\times10^{-7}$
7	2.0	30	.761	$.5\times10^{-7}$
8	2.0	30	.752	$.6\times10^{-7}$
9	2.0	30	.752	$.6\times10^{-7}$
10	2.0	30	.742	$.9\times10^{-7}$
11	2.0	30	.760	$.5\times10^{-7}$
12	2.0	30	.770	$.3\times10^{-7}$
13	2.0	30	.550	$.2\times10^{-3}$
14	2.0	30	.753	$.6\times10^{-7}$
15	2.0	50	*a*. 590	$.4\times10^{-4}$
15	15.0	30	.565	$.1\times10^{-3}$
16	.1	50	.627	$.9\times10^{-5}$
16	2.0	50	*a*. 648	$.4\times10^{-5}$
16	5.0	50	.642	$.5\times10^{-5}$
16	10.0	50	.623	$.1\times10^{-4}$
16	15.0	30	.628	$.9\times10^{-5}$
17	.1	50	.619	$.1\times10^{-4}$
17	2.0	50	*a*. 605	$.2\times10^{-4}$
17	5.0	50	.596	$.3\times10^{-4}$
17	10.0	50	.582	$.5\times10^{-4}$
17	25.0	50	.582	$.5\times10^{-4}$
18	2.0	50	.704	$.4\times10^{-6}$
18	15.0	50	.710	$.3\times10^{-6}$
19	2.0	50	.638	$.6\times10^{-5}$
20	.1	50	.638	$.6\times10^{-5}$
20	2.0	50	*a*. 630	$.8\times10^{-5}$
20	5.0	50	.633	$.7\times10^{-5}$
20	10.0	50	.628	$.9\times10^{-5}$
20	15.0	30	.633	$.7\times10^{-5}$
21	5.0	100	.605	$.2\times10^{-4}$
22	2.0	50	.605	$.2\times10^{-4}$
23	5.0	50	.875	$.5\times10^{-9}$
24	5.0	50	.898	$.2\times10^{-9}$

[a] Average of several determinations.

It is also evident from the data presented in Table II that there is a considerable range in the H-ion concentration of the soil suspensions. A number of soils known to be fertile show strikingly similar reactions, slightly alkaline, as indicated by an H-ion concentration of slightly less than 1×10^{-7}. Alkali soils, presumably containing sodium carbonate, show alkalinity corresponding to an H-ion concentration of 0.2×10^{-9}.

On the other hand, certain of the soils gave an H-ion concentration as high as 0.2×10^{-3}, which indicates considerable intensity of acidity.

Any ultimate conclusions regarding soil fertility must presuppose a knowledge of the composition of the soil solution. At present our methods do not enable us to study the soil solution itself, and consequently all deductions in regard to it must be purely inferential. In order to approximate the conditions existing in the soil solution, the ratio of water to soil in several cases was reduced as far as the method would permit. An inspection of the data indicates comparatively insignificant changes in the H-ion concentrations when widely varying proportions of water to soil are used. Most of these small fluctuations can be ascribed to the limitations of the apparatus. Hence, it is reasonable to assume that the H-ion concentrations of the soil suspensions approximate those of the soil solutions. This argument is theoretically sound, since in all the larger proportions of soil employed it is probable that the solution is saturated with respect to the acid-forming constituents.

RELATION OF HCO_3^-, CO_3^-, AND CO_2 TO SOIL REACTIONS

At present it has been found impracticable to simulate exactly the CO_2 equilibria existing under field conditions. In the case of several acid soils the partial saturation of the soil suspensions with CO_2 gas did not alter the reaction appreciably. Hence, it is quite possible that the CO_2 content of the soil solution may not materially modify the above conclusions regarding the magnitude of the H-ion concentration in acid soils. In other words, the soil acids, whether organic acids or acid silicates, are the chief factors determining the reaction.

On the contrary, the reaction of alkaline soils depends in large measure upon the equilibria between CO_2 gaseous, CO_2 dissolved, CO_3 ion, and HCO_3 ion.[1] From these considerations it might be predicted that the reaction of most alkaline soils is the resultant of the equilibria existing between HCO_3, CO_3, Ca ions and dissolved CO_2 in contact with the CO_2 of the soil atmosphere. From the work of Cameron and Bell (6) and Johnson (19) it seems proper to infer that HCO_3^- in this class of soils largely determines the reaction.

The application of the electrometric method to solutions or suspensions whose reaction depends upon the HCO_3 ion requires great precaution to prevent the decomposition of HCO_3^-, with a loss of CO_2. This reaction is slow, but there will be a gradual increase in the alkalinity of the solution as a result of the production of CO_3 ions. In the present work the shaking method by Gillespie (13) was employed and the decomposition of HCO_3^- largely avoided, although the results may tend to be slightly high.

[1] For a detailed discussion of this matter the reader is referred to Cameron and Bell (6) and to Johnson (19).

As a means of comparison the OH-ion concentration of solutions saturated with $Ca(HCO_3)_2$ and $CaCO_3$ were determined with an exactly similar technique. $Ca(HCO_3)_2$ gave a value for OH^- of 0.5×10^{-7} and for $CaCO_3$ 0.3×10^{-9}. The figure for $Ca(HCO_3)_2$ is almost identical with those obtained for the alkaline soils. This is in keeping with the theoretical considerations advanced above. T. Saidel (29) determined the OH-ion concentration of several soil extracts after prolonged boiling. His values approximate that given by $CaCO_3$, which is distinctly higher than the normal OH^- concentration for soils of this class.

At first attempts were made to estimate the H-ion concentration of filtered soil extracts. Serious difficulty was encountered in this procedure, in that the nitrates present were slowly reduced by the hydrogen gas in contact with platinum black. Obviously this reduction of nitrates would result in the production of NH_3 and a residue of fixed alkali, with a corresponding increase in the concentration of OH ions. While the reduction of nitrates does occur to some extent in soil suspensions, yet in this case the results are not appreciably changed. This is accounted for by the fact that the acid soils contain a large excess of potential acidity, while the absolute amounts of alkalinity produced are exceedingly minute. Therefore, equilibrium would be immediately restored without sensibly affecting the true H-ion concentration. As evidence thereof, the voltmeter readings for acid soils became constant within a few minutes and remained constant for an indefinite time.

In order to determine whether the reduction of nitrates gave rise to appreciable errors in the readings for alkaline soils, the OH^- concentration of a $Ca(HCO_3)_2$ solution was measured in the presence of large amounts of $NaNO_3$. No disturbance of the equilibria was noted under the conditions of the experiment. It has been suggested that the use of palladium or iridium, instead of platinum, for coating the electrodes would practically prevent the reduction of nitrates, but the authors have no experimental data upon this point.

While the reduction of nitrates prevents the use of the hydrogen electrode with extracts of acid soils, it is not believed that this factor would cause appreciable errors in the case of nutrient solutions containing nitrates. A nutrient solution would usually have a considerable potential acidity or alkalinity—for example, the unhydrolyzed or undissociated fraction of alkaline phosphates—so that constant results could be obtained, just as with soil suspensions. Moreover, by use of the shaking method described by Michaelis (21) the reduction of NO_3^- may be reduced to a minimum, but even this slight production of alkali causes serious error in soil extracts, where the total quantity of acid present is exceedingly small.

EFFECT OF GRINDING SOILS ON THE HYDROGEN-ION CONCENTRATION OF THEIR SUSPENSIONS.

The determination of the lime requirement of soils by the usual methods depends upon several factors. Among these factors the fineness of division has recently received some attention. Cook (9) found that in certain soils the lime requirement by the Veitch method increased with grinding, while Brown and Johnson (4) obtained opposite results working with another group of soils. Several of the soils already described were ground to pass a 200-mesh sieve. Table III shows the H-ion concentration of suspensions made from the ground soil.

TABLE III.—*Hydrogen-ion concentrations in suspensions of soil ground to pass through a 200-mesh sieve*

Soil No.	Quantity of soil.	Water.	Readings on voltmeter.	H-ion concentration (gram molecules per liter).
	Gm.	*C. c.*		
15	0.01	50	0.629	0.8×10^{-5}
15	.10	50	.617	$.1\times10^{-4}$
15	.50	50	.632	$.7\times10^{-5}$
15	1.0	50	[a].612	$.2\times10^{-4}$
15	2.0	50	[a].593	$.4\times10^{-4}$
15	5.0	50	[a].560	$.1\times10^{-3}$
15	10.0	50	.577	$.7\times10^{-4}$
16	1.0	50	[a].653	$.3\times10^{-5}$
16	5.0	50	.625	1.0×10^{-5}
17	.01	50	[a].615	$.1\times10^{-4}$
17	1.0	50	.624	$.1\times10^{-4}$
17	5.0	50	[a].597	$.3\times10^{-4}$
20	2.0	50	[a].623	$.1\times10^{-4}$
19	1.0	30	.646	$.4\times10^{-5}$
19	2.0	50	[a].634	$.7\times10^{-5}$
18	.5	50	.750	$.7\times10^{-7}$
18	2.0	50	.748	$.7\times10^{-7}$
18	5.0	50	.762	$.4\times10^{-7}$

[a] Average of several determinations.

By comparing Table III with Table II it will be seen that with the exception of soil 18 grinding did not materially alter the H-ion concentration of the soil suspensions. These remarks bear no reference to the lime requirement, which will be discussed later, but apply only to intensity of acidity. Apparently the anomalous behavior of soil 18 may be explained on the supposition that the interior cores of the soil particles are of a different composition from the exterior, partially weathered layers. This hypothesis may also account for the findings of Brown and Johnson (4).

The data of Table III also corroborate in the main those presented in Table II in reference to the slight fluctuations in H-ion concentration due to varying the proportion of soil to water.

EFFECT OF HEATING ON THE HYDROGEN-ION CONCENTRATION

In order to ascertain the effect of heating upon the H-ion concentration, several soils were subjected to the heating treatments indicated in Table IV.

TABLE IV.—*Effect of heating on H-ion concentration of soil suspensions*

Soil No.	Quantity of soil.	Water.	Method of heating.	Time of heating.	Voltmeter reading.	H-ion concentration (gram molecules per liter).
	Gm.	*C. c.*		*Hours.*		
15.....	2	50	Oven 140° C.....	3	0.578	0.6×10^{-4}
16.....	2	50	.....do.....	3	.621	$.1\times10^{-4}$
17.....	2	50	.....do.....	3	.583	$.5\times10^{-4}$
17.....	5	50	Muffle below red heat.	1	.652	$.3\times10^{-5}$
17.....	2	50	.....do.....	1	.730	$.1\times10^{-6}$
17.....	2	50	Blasted.....	1	.812	$.6\times10^{-8}$
19.....	2	50	Oven 140° C.....	3	.603	$.2\times10^{-4}$
19.....	2	50	.....do.....	3	.598	$.3\times10^{-4}$
20.....	2	50	.....do.....	3	.672	$.2\times10^{-5}$
20.....	2	50	Muffle below red heat.	3	.678	$.1\times10^{-5}$
20.....	2	50	Blasted.....	3	.803	$.8\times10^{-8}$

The data of Table IV confirm the views of Connor (8), in that the intensity of acidity decreases when the soils are heated at high temperatures. The insufficiency of the data concerning heating at 140° C. does not admit of positive conclusions, though there is indication that the H-ion concentration may be slightly increased by this treatment.

ESTIMATION OF THE LIME REQUIREMENT BY THE ELECTROMETRIC METHOD

The rational treatment of acid soils requires that sufficient lime be added to bring the soil to a neutral or slightly alkaline reaction. The attainment of this point may be definitely determined by the method described in this paper. Many empirical methods have been suggested for the determination of the lime requirement, but the inaccuracy of these methods is indicated by the enormous variations in results, as shown in the comparative tests reported by Ames and Schollenberger (1) and others. These findings are also confirmed by data obtained in this laboratory.

An attempt was made to determine more precisely the lime requirement of soils by a method of electrometric titration with calcium hydroxid, $Ca(OH)_2$, in which a standard calcium-hydroxid solution was added to the soil suspensions until a definite alkaline reaction was obtained. The data were supplemented by pot and beaker studies. Tables V and VI present the results of these experiments.

TABLE V.—*Titration of soil suspensions with calcium hydroxid for lime requirement*

Soil No.	Quantity of soil.	Water.	Quantity of calcium hydroxid added (calculated to calcium carbonate).		H-ion concentration.				Time of titration.
					Original.		After titration.		
			Entire quantity.	Per gram of soil.	Voltmeter reading.	Gram molecules per liter.	Voltmeter reading.	Gram molecules per liter.	
	Gm.	*C. c.*	*Gm.*	*Gm.*					*Hours.*
15.....	5	50	0.0085	0.0017	0.590	0.4×10^{-4}	0.775	0.2×10^{-7}	3
15.....	5	50	.0128	.0026	.590	$.4\times10^{-4}$	.796	$.1\times10^{-7}$	44
16.....	2	50	.0054	.0027	.654	$.3\times10^{-5}$	.761	$.4\times10^{-7}$	26
17.....	2	50	.0061	.0030	.611	$.2\times10^{-4}$	.759	$.5\times10^{-7}$	26
17.....	2	50	.0070	.0035	.599	$.3\times10^{-4}$	.757	$.5\times10^{-7}$	5
19.....	2	50	.0081	.0040	.638	$.6\times10^{-5}$	.770	$.3\times10^{-7}$	25
20.....	2	50	.0053	.0026	.618	$.1\times10^{-4}$	.754	$.6\times10^{-7}$	3
20.....	2	50	.0047	.0023	.623	$.1\times10^{-4}$	.757	$.5\times10^{-7}$	5
18.....	2	50	.00068	.00034	.707	$.4\times10^{-6}$	.758	$.5\times10^{-7}$	20

TABLE VI.—*Results of beaker and pot studies*

Soil No.	Soil in beaker or pot.	Total quantity of calcium carbonate added.	Calcium carbonate per gram of soil.	Treated soil tested.	Water.	Reaction of treated soil.		Remarks.
						Readings on voltmeter.	H ion (gram molecules per liter).	
	Gm.	*Gm.*	*Gm.*	*Gm.*	*C. c.*			
15..	200	0.200	0.001	5	50	0.664	0.2×10^{-5}	Incubated 7 days at 30° C.
15..	200	.400	.002	5	50	.799	$.9\times10^{-8}$	Do.
15..	11,200	10.0	.0009	5	50	.630	$.8\times10^{-5}$	In jar 2 months.
15..	11,200	15.0	.0013	5	50	.733	$.1\times10^{-6}$	Do.
16..	50	.135	.0027	5	50	.752	$.6\times10^{-7}$	Incubated 7 days at 30° C.
17..	25	.090	.0036	5	50	.728	$.2\times10^{-6}$	Do.
17..	50	.250	.0050	5	50	.777	$.2\times10^{-7}$	Do.
18..	50	.017	.00034	5	50	.740	1.0×10^{-7}	Do.
19..	25	.100	.0040	5	50	.755	$.5\times10^{-7}$	Do.
20..	25	.065	.0026	5	50	.780	$.2\times10^{-7}$	Do.

Such a method is logically adapted to obtain the information necessary for the proper adjustment of the soil reaction by the addition of lime. There are, however, certain difficulties met with in its application to soils. One of the chief obstacles is due to the relative insolubility of the acid-forming constituents of soils, which prevents a rapid attainment of equilibrium. This drawback can probably be overcome by the use of a shaking machine. Another but presumably minor source of error lies in the loss of CO_2 from the soil suspension, as previously mentioned.

In order to determine whether the titrations with calcium hydroxid might serve as a guide for the application of lime necessary to produce a neutral or slightly alkaline reaction several beaker and pot studies were undertaken. A reference to Tables V and VI shows that in these soils approximate neutrality resulted from the admixture of calcium carbon-

ate with the soil in amounts indicated by the titrations. While these data are not extensive, a valuable correlation is suggested.

So far as the writers are aware, this is the first time that this electrometric titration has been applied to soil studies. It is believed that with further work a valuable means may be developed for the more exact determination and adjustment of soil reaction. Hence, this method may be extremely useful in the accurate control of soil reaction in many field and pot experiments. The somewhat complicated nature of the apparatus and the time involved would doubtless militate against its general adoption for routine analyses.

EFFECT OF THE ADDITION OF NEUTRAL SALTS ON THE HYDROGEN-ION CONCENTRATION OF SOIL SUSPENSIONS

The effect on the H-ion concentration of soil suspensions produced by the addition of neutral salts is a matter of considerable theoretical interest and of practical importance. Such data may be of significance in their relation to the application of soluble fertilizing salts and to the various lime-requirement methods dependent upon treatments with solutions of potassium nitrate. The desirability of similar measurements as correlated with the effects of soluble salts on the physical condition of soils has already been suggested by one of the authors (31). The bearing of the results upon adsorption phenomena will be discussed in a later section of this paper.

Table VII records the changes in H-ion concentration of various soil suspensions when treated with different neutral salts:

TABLE VII.—*Effects of neutral salts on H-ion concentration of soil suspensions*

Soil No.	Quantity of soil.	Water.	Salt added.	Quantity of salt.	Original soil.		Treated soil.	
					Voltmeter reading.	H-ion (gram molecules per liter).	Voltmeter reading.	H-ion (gram molecules per liter).
	Gms.	*C. c.*		*Gm.*				
16..	2	30	Potassium chlorid.	1	0.628	0.9×10^{-5}	0.582	0.5×10^{-4}
16..	2	30	Sodium chlorid...	1	.628	$.9\times10^{-5}$	.572	$.8\times10^{-4}$
16..	2	30	Barium chlorid...	1	.628	$.9\times10^{-5}$	.555	$.2\times10^{-3}$
20..	2	30	Potassium chlorid.	1	.639	$.5\times10^{-5}$	.575	$.7\times10^{-4}$
20..	2	30	Sodium chlorid...	1	.639	$.5\times10^{-5}$	.568	1.0×10^{-4}
20..	2	30	Barium chlorid...	1	.639	$.5\times10^{-5}$	.564	$.1\times10^{-3}$
15..	2	30	Potassium chlorid.	1	.598	$.3\times10^{-4}$	.548	$.2\times10^{-3}$
15..	2	50	Sodium chlorid...	0.5	.598	$.3\times10^{-4}$	.551	$.2\times10^{-3}$
15..	2	30	Barium chlorid...	1	.598	$.3\times10^{-4}$	.535	$.3\times10^{-3}$
18..	2	30	Potassium chlorid.	1	.690	$.7\times10^{-6}$	.614	$.2\times10^{-4}$
18..	2	30	Sodium chlorid...	1	.690	$.7\times10^{-6}$	.615	$.2\times10^{-4}$
18..	2	30	Barium chlorid...	1	.690	$.7\times10^{-6}$	.590	$.4\times10^{-4}$
14..	2	30	Potassium chlorid.	1	.753	$.6\times10^{-7}$	.662	$.2\times10^{-5}$
14..	2	30	Sodium chlorid...	1	.753	$.6\times10^{-7}$	.672	$.2\times10^{-5}$
14..	2	30	Barium chlorid...	1	.753	$.6\times10^{-7}$	.654	$.3\times10^{-5}$
1...	2	50	Potassium chlorid.	0.5	.763	$.4\times10^{-7}$	.763	$.4\times10^{-7}$

A study of the foregoing data makes it evident that in all cases there has been a distinct increase in the H-ion concentration, when either potassium chlorid, sodium chlorid, or barium chlorid was added to the suspension in the quantities indicated. The increase does not vary greatly for the three salts used, but on the whole the barium chlorid has a somewhat greater effect. In soil 14 we have an interesting case in which a change of reaction from alkaline to acid has taken place, as a result of the addition of neutral salts of sodium, potassium, or barium. It is obvious that such a soil would probably be adjudged acid by the potassium-nitrate method, although its normal reaction is, in fact, slightly alkaline. In a more normal type of soil (No. 1), however, no change in the reaction is found. The use of calcium chlorid in the above experiment was found to be impracticable on account of the difficulty in obtaining a perfectly neutral salt.

ADSORPTION OF OH IONS BY SOILS IN SUSPENSIONS OF VARIOUS BASES

So far the H-ion concentration of soil suspensions, the factors affecting it, and the possible use of an electrometric titration method for determining the lime requirement have been the chief topics considered. We shall now consider another phase of the general problem, involving the question of the adsorption of OH ions by the soil. The hydrogen electrode has proved useful for this purpose.

Changes of the OH-ion concentration of soil suspensions were measured when varying quantities of different hydrates were added. In addition the removal of Ca from a solution of hydrate in contact with two of the soils studied was noted. The data obtained from these experiments are incorporated in Tables VIII and IX.

To suspensions of soils that pass through a 200-mesh sieve the hydrates were added, a small portion at a time, until the neutral point was just passed; then further additions of hydrate were made until an arbitrarily selected OH-ion concentration was maintained over a considerable period of time, as noted in Table VIII. After each addition of the hydrate the soil suspension was given a prolonged shaking. The bases added have been calculated for convenience of comparison to the equivalent OH expressed in grams. The above data enable us to estimate the approximate quantity of OH ions removed from the solution by the soil, and in two cases where calcium hydroxid was added the removal of Ca has also been determined by the usual analytical method.

TABLE VIII.—*Results of titrations of ground (200-mesh sieve) soil with various bases*

Soil No.	Quantity of soil.	Water.	Base.	Weight of OH.	OH per gram of soil.	Time of titration.	Original OH-ion concentration (gram molecules OH per liter).	OH-ion concentration at approximate neutrality (gram molecules OH per liter).	Base added beyond neutral point (weight of OH).	OH per gram soil.	Time of titration.	Final OH-ion concentration (gram molecules OH per liter).
	Gm.	*C.c.*		*Gm.*	*Gm.*	*Hrs.*			*Gm.*	*Gm.*	*Hr.*	
15..	0.5	50	Calcium hydroxid.	0.00038	0.00076	1.5	0.9×10^{-9}	0.8×10^{-7}	0.00006	0.00012	3	1.9×10^{-7}
15..	10.0	50	.....do.....	.00798	.00079	2.0	$.9\times10^{-10}$	2.0×10^{-7}	.00088	.00009	96	2.9×10^{-7}
15..	1.0	50	.....do.....	.....	.....	.....	3.9×10^{-10}	.....	[a].00211	[a].00211	26	$.9\times10^{-4}$
15..	1.0	50	Sodium hydroxid.	.....	.....	.....	3.6×10^{-10}	.....	[a].00207	[a].00207	26	2.2×10^{-4}
15..	2.0	50	Calcium hydroxid.	.00163	.00081	.....	2.1×10^{-10}	4.3×10^{-8}	.00014	.00007	3	2.7×10^{-7}
15..	2.0	50	Sodium hydroxid.	.00131	.00065	24.0	1.5×10^{-10}	$.7\times10^{-7}$	.00026	.00013	27	2.4×10^{-7}
15..	5.0	50	Calcium hydroxid.	.00429	.00085	6.0	$.7\times10^{-10}$	$.9\times10^{-7}$	.00029	.00006	50	1.1×10^{-7}
15..	5.0	50	Sodium hydroxid.	.00350	.00070	3.0	4.2×10^{-11}	1.1×10^{-7}	.00052	.00010	27	1.2×10^{-7}
16..	5.0	50	.....do.....	.00560	.00112	20.0	$.6\times10^{-9}$	$.6\times10^{-7}$	.00105	.00021	42	1.7×10^{-7}
16..	1.0	50	Calcium hydroxid.	.00103	.00103	24.0	2.0×10^{-9}	$.6\times10^{-7}$	.00340	.00340	48	4.2×10^{-5}
16..	1.0	50	Barium hydroxid.	.00099	.00099	0.5	1.0×10^{-9}	1.9×10^{-7}	.00280	.00280	30	3.8×10^{-5}
16..	1.0	50	Sodium hydroxid.	.00105	.00105	3.0	2.0×10^{-9}	1.5×10^{-7}	.00263	.00263	30	4.2×10^{-5}
16..	1.0	50	Potassium hydroxid.	.....	.....	.....	1.9×10^{-9}	.....	[a].00324	[a].00324	24	5.7×10^{-5}
17..	1.0	50	Calcium hydroxid.	.00088	.00088	2.5	5.8×10^{-10}	1.9×10^{-7}	.00474	.00474	30	$.7\times10^{-4}$
17..	1.0	50	Barium hydroxid.	.00066	.00066	1.5	6.3×10^{-10}	1.7×10^{-7}	.00406	.00406	30	$.9\times10^{-4}$
17..	5.0	50	Calcium hydroxid.	.00650	.00130	1.0	2.0×10^{-10}	$.8\times10^{-7}$	.00800	.00160	22	1.3×10^{-6}
17..	5.0	50	Sodium hydroxid.	.00438	.00087	4.0	2.2×10^{-10}	$.8\times10^{-7}$	.00526	.00105	110	1.7×10^{-6}
19..	2.0	50	Calcium hydroxid.	.00306	.00153	7.0	$.9\times10^{-9}$	$.9\times10^{-7}$	.01010	.00505	30	4.2×10^{-5}
19..	2.0	50	Barium hydroxid.	.00330	.00165	21.0	$.9\times10^{-9}$	$.8\times10^{-7}$	.00858	.00429	30	3.5×10^{-5}
19..	2.0	50	Sodium hydroxid.	.00207	.00103	7.0	$.7\times10^{-9}$	1.0×10^{-7}	.00350	.00175	30	4.1×10^{-5}
19..	2.0	50	Potassium hydroxid.	.00198	.00099	24.0	1.3×10^{-9}	$.7\times10^{-7}$	.00524	.00262	30	3.8×10^{-5}
20..	2.0	50	Calcium hydroxid.	.00118	.00059	4.0	5.8×10^{-10}	5.9×10^{-8}	.00446	.00223	28	4.8×10^{-5}
20..	2.0	50	Sodium hydroxid.	.00091	.00045	3.5	6.3×10^{-10}	$.7\times10^{-7}$	.00249	.00125	28	3.1×10^{-5}

[a] Includes base added to neutralize acid.

TABLE IX.—*Removal of calcium from solution of calcium hydroxid by soils*

Experiment No.	Soil No.	Weight of soil.	Water.	Calcium added.	Calcium recovered in solution.	Calcium removed from solution.	
		Gm.	*C.c.*	*Gm.*	*Gm.*	*Gm.*	*P. ct.*
1	17	20	50	0	0.0003	.....	.....
2	17	20	50	.0055	.0007	0.0048	87
3	17	20	50	.0137	.0023	.0114	83
4	17	20	50	.0192	.0053	.0139	73
5	17	20	50	.0220	.0067	.0153	70
6	17	20	50	.0247	.0089	.0158	64
7	20	20	50	.....	.0003	.....	.....
8	20	20	50	.0022	.0003	.0019	86
9	20	20	50	.0038	.0003	.0035	92
10	20	20	50	.0055	.0005	.0050	91
11	20	20	50	.0066	.0005	.0061	92
12	20	20	50	.0082	.0009	.0073	89

When 0.1 mgm. of OH as calcium hydroxid is added to 50 c. c. of distilled water, there is produced an OH-ion concentration of 3.8×10^{-5} gram molecules per liter. To reach this same concentration of OH ions in the presence of the soil requires the addition of a much greater quantity of hydrate, whether of sodium, calcium, barium, or potassium. The difference between the quantity of hydrate necessary to add to distilled water to obtain the concentration fixed upon and that required when the soil is present gives an index of the amount of hydroxyl ions removed from the ionic equilibrium. In the case of acid soils it is probable that until the neutral point is reached the removal of OH ions can be accounted for by the reaction between the added OH ions and H ions derived from the soil acids. Beyond the neutral point, however, another type of reaction must necessarily be involved. For example, soil 16 required the addition of 1.0 mgm. of OH as calcium hydroxid per gram of soil to give the suspension an OH-ion concentration of 0.6×10^{-7}, while the second point, representing a concentration of 4.2×10^{-5} gram molecules of OH ions per liter, required the further addition of 3.4 mgm. of OH per gram of soil. By subtracting the quantity of OH required to reach the same point when added to distilled water, it is evident that 3.3 mgm. of OH have been removed by some mechanism not associated with the neutralization of acid. The nature of the latter type of reaction has been the subject of much conjecture; but before entering upon a detailed discussion of this matter it is desirable to point out certain other relations which may be derived from a further study of the data presented above. These are concerned with the comparison of the amounts of OH ions removed from solutions of the various bases, when added in combination with different positive ions. In general, it may be said that to bring about this higher OH-ion concentration in the soil suspension requires a larger equivalent of calcium hydroxid and barium hydroxid than of sodium hydroxid or potassium hydroxid. As a striking instance of this, soil 19 may be cited. In order to assure a reasonable degree of validity for these comparisons, they have been made, in accordance with Hanley's (14) suggestions, under conditions such that the suspended soil particles were in equilibrium with solutions of the same concentration. It is difficult to draw definite conclusions in regard to the relative quantities of bases required to produce neutrality, but it may be said that the equivalent quantities are of approximately the same magnitude. Exact equivalents would hardly be expected on account of various side reactions involving the interchange of bases.

The interesting observation recorded in Table VII that barium chlorid when added to soils brings about a slightly greater acidity than when sodium or potassium chlorid is added may possibly be correlated with the fact that a greater quantity of OH is removed by the soil when added as barium hydroxid than when added as sodium or potassium hydrate. This agrees with certain contentions of Parker (24).

Many investigators consider soil acidity as being primarily related to adsorptive phenomena. Cameron (5) has explained the reddening of blue litmus paper by many soils as due to selective absorption of the base from the litmus base and does not correlate the reddening of the litmus paper with the presence of soluble acid except in very rare cases. Harris (15) also ascribes soil acidity to the selective adsorption of bases by the soil. Bogue (3) and others have expressed a similar opinion concerning soil acidity. The phenomena of adsorption, as is well known, has also been invoked to explain the fixation of various ions by the soil.

Another view of soil acidity attributes it to an exchange of bases in which a weak base, as aluminium, has been replaced by a strong base such as potassium. The resulting hydrolysis of the aluminium salts produces an acid reaction in the solution. This view has been advanced most recently by Rice (26), Conner (8), Loew (20), Daikuhara (10), and Veitch (35). This is a plausible explanation for the acidity of soils which have been treated with salts, although it does not indicate the reaction of the soil previous to the salt treatments. The results already presented in regard to the addition of salts show that in the case of acid soils this treatment markedly increased the H-ion concentration in every case. One slightly alkaline soil was also found to give an acid reaction after the salt treatments. These data are in direct accord with the accumulated evidence concerning the acidity developed when a strong base reacts with the soil constituents replacing a weak base. In the light of these observations it is quite unnecessary to have recourse to adsorption theories to account for the acidity of many salt-treated soils, even though the soils originally might have had an alkaline reaction. This is illustrated in the case of soil 14, already cited. Obviously, the principal reactions involved in producing the acidity in such cases are of a chemical rather than of a physical nature. The importance of such an exchange of bases is emphasized by the work of Sullivan (32), Rice (26), and Conner (8).

Truog (33), Hanley (14), Gillespie (13), and Loew (20) have recognized that the acidity of a soil is due to the presence of soluble acids. The experimental evidence reported in this paper has brought the writers to a similar conclusion, in which case it is not necessary to associate soil acidity with physical adsorption.

A great many obscure phenomena in the fields of biochemistry and soil chemistry, as well as many others, have been classified under the indefinite terms "absorption" and "adsorption." The indiscriminate use of these terms has not served to clarify the problem involved. Thus, the fixation of plant foods by soils has been explained by some investigators entirely on the basis of physical adsorption. Frequently, the importance of the chemical exchange of bases has been disregarded. Moreover, the possibility of addition compounds as suggested by Sullivan (32) has not received sufficient consideration. The results given in Table VII show

that the OH ions of a solution of calcium hydroxid have been removed in large measure from the solution by the soil, while those in Table VIII demonstrate a simultaneous loss of calcium from the solution. Evidently both the Ca and OH ions have been removed to a notable extent from the solution by the soil. Two explanations of these phenomena suggest themselves. One explanation would assume a condensation of the calcium hydroxid as a whole on the surfaces of the soil particles. The other, in accordance with the ideas of Sullivan (32), Van Bemmelen (34), and Lemberg (32, p. 20–23), considers that the calcium hydroxid has been chemically united with some of the soil constituents, forming direct addition compounds. In view of the evidence embodied in the literature, the second theory seems the more tenable. In many ways the chemical explanation appears to be more logical in accounting for the fixation of bases by soils.

Moreover, the theories which regard adsorption in soils as a chemical phenomenon have received an element of support in the proposed chemical structure of the silicates. Clarke and Steiger (7) have shown that the composition of the silicates is such that it frequently admits of an exchange of bases. This has also been recognized by soil chemists. Likewise, from the structural formulæ for silicates proposed by the above investigators, it is evident that acid salts of the various silicic acids might contain replaceable hydrogen. Loew (20) attributes the acidity of certain Porto Rican soils to the presence of acid silicates. As previously mentioned, Sullivan (32) has also pointed out that a basic hydrate may form an addition compound with silicates.

The theory of selective adsorption of a single ion which has been advanced by various investigators is in its final analysis not entirely comprehensible. One objection to this theory lies in the disregard of the ionic equilibrium. For example, it is frequently assumed that from a solution of a neutral salt one ion may be withdrawn by a colloid independently of its equivalent, oppositely charged ion. Thus if K^+ be selectively removed from a solution of potassium chlorid (KCl), then the above assumption may be diagramatically represented as follows:

$$\begin{array}{l} (\text{Colloid}-\text{K})+\overset{-}{\text{Cl}}+\text{H}_2\text{O} \\ \qquad\qquad\quad \uparrow\downarrow \\ \qquad\qquad\quad \overset{+}{\text{H}}+\overset{-}{\text{OH}} \end{array}$$

Obviously, such a system is electrically unbalanced. In order to meet this difficulty, the electrical double-layer theory of Helmholtz has been proposed. A modification of this theory has been especially urged by Billitzer (2). In its simplest form the electrical double-layer theory assumes that one ion may become more closely associated with a colloidal particle than the oppositely charged ion. The force which binds the ion to the colloid is not well understood, but there is some justification for believing that the most closely associated ion imparts its

charge to the colloidal particle. The charge thus produced on the surface of the particle is balanced by oppositely charged ions in the immediate sphere of attraction. In this manner the conditions of electrical equilibrium are accounted for.

Parker (24) has avoided some of the difficulties involved in explaining the electrical equilibria on the assumption that one ion alone is removed by a colloid. He claims that potassium chlorid in aqueous solution is hydrolyzed, yielding hydrochloric acid and potassium hydroxid. The latter is then considered to be withdrawn from the solution as a whole. Obviously, this does not explain the mechanism by which the potassium hydroxid is combined with the colloid, although it does take into account the electrical equilibria and the presence of acid in the solution. It does not, however, exclude the possibility of a direct chemical addition product of potassium hydroxid with some of the soil constituents.

Either of the last two theories may account for the presence of acid in the filtrate or supernatant liquid derived from a colloid in contact with a salt solution. Whichever explanation may be preferable, for practical purposes the final result is the same—that is, the colloid has retained equivalent quantities of negative and positive ions, and chemically equivalent quantities remain in solution.

Since these theories of adsorption necessitate the removal of both positive and negative ions, the usefulness of the idea of selective adsorption as applied to many types of soil reactions may be questioned, especially in view of the possibility that chemical reactions, at least to a considerable extent, may account for the observed phenomena.

This statement is not to be construed as denying the probability of a partial condensation of a chemical compound on solid surfaces. A clear exposition of this type of phenomena is given by Patten (25). The magnitude of such condensations is exceedingly variable, for it depends upon the physical conditions of the particular system, and, hence, it is difficult to estimate its significance in any specific instance. Indeed, Robertson (27) is inclined to believe that surface condensation accounts for only a small portion of the total amount of substance combined with the adsorbing body. In his opinion most reactions designated by the terms "absorption" and "adsorption" obey the usual laws formulated for chemical reactions. The influence of surface in accelerating and possibly changing the nature of these reactions is not denied. This solution of the problem, as stated by Robertson, is not admitted by certain other investigators, notably Wolfgang Ostwald (23). Apparently, no final decision on this matter has been reached by physical chemists.

It has been the aim of the present paper to set forth certain results and methods of investigation which may serve to throw additional light on some of the unsolved problems of soil fertility. Especially has the method of attack made possible an investigation of the important ques-

tions of soil acidity and adsorption on a new basis. The evidence has been confirmatory of the view that soil acidity is fundamentally dependent upon the equilibria of reactions yielding an excess of H ions, and is not necessarily related to the various phenomena grouped under the terms "absorption" and "adsorption." Although the literature concerning soils constantly refers to "absorption" and "adsorption," yet no very concise meaning has been attached to these terms. In fact, there is quite as much evidence in favor of a chemical, as opposed to a physical, interpretation of such phenomena. For these reasons a more critical examination of this field would be a welcome addition to agricultural science.

DESCRIPTION OF EXPERIMENTAL APPARATUS

The apparatus used in determining the H-ion concentration is similar to that described by Hildebrand (17), with such modifications as are necessary or convenient for purposes of soil investigations (fig. 1). Precise methods for determining small differences of potential have frequently been described, but the use of an elaborate potentiometer system is neither practicable nor necessary in work with soils or nutrient solutions. Obviously measurements of physical-chemical exactitude are useless unless all the factors involved are capable of equally exact control, which is not possible with most substances of agricultural interest. For practical application, therefore, the voltmeter method of Hildebrand is entirely adequate in point of accuracy and at the same time rapid and convenient.

For information in regard to the physical-chemical principles underlying the method, the reader is referred to Hildebrand (17), Michaelis (21), Itano (18), or to textbooks on electrochemistry. Very convenient tables for the transformation of voltmeter readings into H- and OH-ion concentrations have been prepared by Schmidt (30). It is deemed desirable, however, to present here certain details of the apparatus and method of procedure, since these are not easily available to the general worker in agricultural laboratories. Moreover, measurements with soil suspensions require special precautions, to avoid otherwise very misleading results.

The arrangement of the apparatus and method of wiring are shown in figure 1. The entire system includes the following pieces of apparatus: Dry cell, two rheostats of 40 ohms resistance, with sliding contacts; Weston voltmeter, 0 to 1.2 volts; Leeds and Northrup portable galvanometer, sensitivity of 1 megohm; contact key; calomel cell, hydrogen-electrode vessel; hydrogen generator, with purifying tube, wash bottles, and rheostat or lamp board connected with direct current.

A convenient Cottrell hydrogen electrode, designed by the Department of Chemistry, University of California, consists of a glass tube of about 1 cm. in diameter and 15 cm. in length, in the end of which is sealed a

cylinder of fine platinum gauze approximately 1 cm. long. Near the upper end of the electrode a branch tube permits the entrance of hydrogen gas. A platinum wire is affixed to the gauze at one end and sealed into a small glass tube at the other. The electrical connection is then

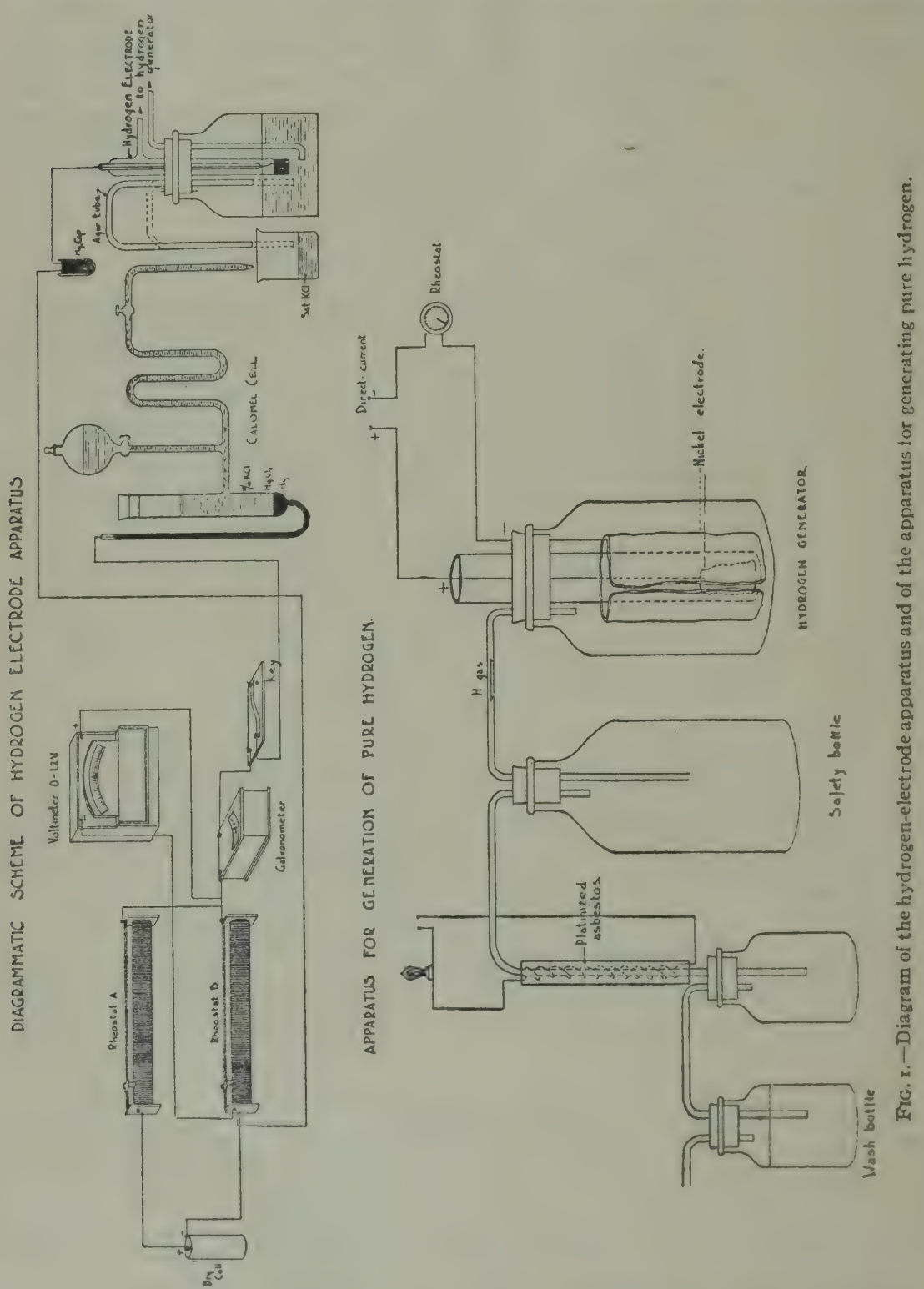

FIG. 1.—Diagram of the hydrogen-electrode apparatus and of the apparatus for generating pure hydrogen.

made by the use of a copper wire and a mercury cup, as shown in figure 1. The electrode must be gas-tight at the top. Platinum black is deposited on the gauze, as described by Ostwald (22). The coating should be renewed occasionally. It is sometimes necessary to clean the plat-

inum between determinations with a little diluted hydrochloric acid, afterward rinsing in many changes of distilled water.

The electrode vessel must be closed to the air. A simple and easily constructed cell may be made from a wide-mouthed bottle of 75 to 100 c. c. capacity. Through holes in the rubber stopper are fitted the hydrogen electrode, an agar connecting tube, a small exit tube, and a tube for saturating the solution with hydrogen when desired. A hole to admit the tip of a burette should also be provided for purposes of titration. The most convenient method of making the liquid connection is by means of bent glass tubes, filled with agar jelly prepared with a saturated solution of potassium chlorid. Only thoroughly washed agar should be used in making the jelly. In order to avoid contact potentials as far as possible connection with the calomel cell is made through a beaker, containing saturated potassium-chlorid solution. The construction of a normal calomel cell is described by Ostwald (22). In the present work *N/10* potassium chlorid was used.

An adequate supply of pure hydrogen is of primary importance, and this is most conveniently obtained from the decomposition of water by means of a direct electric current. Such a generator may easily be prepared by using a large bottle and an inner cylinder made from a wide glass tube as a means of separating the electrodes. The latter may be of nickel, or pure iron and a 25 per cent (by weight) potassium hydroxid solution is convenient as an electrolyte. In order to purify the hydrogen from small quantities of oxygen, it is passed through a long glass tube filled with platinized asbestos. This is heated by a fine nichrome wire wound around the outside of the tube and connected through a lamp with a source of current. To provide a rapid stream of hydrogen requires the consumption of several amperes of current.

EXPERIMENTAL PROCEDURE

After placing the soil suspension in the bottle, hydrogen gas is permitted to flow through the electrode raised above the surface of the liquid for several minutes. The electrode is now lowered until the platinum gauze is partially submerged in the liquid and the exit tube closed. The bottle is now rotated back and forth for several minutes, as originally described by Hasselbach and Gammeltoft (16). The agar tube (at other times kept out of the liquid) is now lowered so that a connection is made with the calomel cell through the beaker of potassium chlorid. The rheostats are adjusted so that no deflection of the galvanometer needle is noted when momentary connection is made by tapping the key. The reading on the voltmeter is then recorded. The procedure is repeated until constant readings are obtained. This occurs in the case of acid soils within a few minutes, but for soils approximately neutral a slightly longer time will be required. In the case of titrations

prolonged shaking is required after each addition of the titrating solution, in order to obtain constant readings. This is due to the slow rate of solubility possessed by the acid constituents of the soil. The use of a mechanical shaking device would doubtless greatly facilitate the operation.

Duplicate determinations on soil suspensions usually agree within 0.01 to 0.02 volt. In any one determination constancy of readings may be obtained to within 0.002 volt. The larger sources of error result from the change in the decomposition of the solution due to reduction of nitrates or the breaking down of the HCO_3 ions. These points have already been thoroughly considered. Robertson (28) and later Desha and Acree (11) have shown the possible interference of certain types of organic matter. Saturation of the electrode with hydrogen before dipping seems to obviate the error, and no difficulty from this source was experienced in the present work.

The use of the modification of Hildebrand's apparatus described in this paper and due observance of the special precautions noted will, it is believed, enable the investigator of soils and plants to obtain valuable information in regard to H-ion concentrations without undue loss of time. In many fields of biochemistry [1] similar methods have been extensively employed during the last few years.

SUMMARY

(1) Soil acidity is due to the presence of an excess of hydrogen ions in the soil solution.

(2) Direct evidence of this fact is given by hydrogen-electrode measurements.

(3) The hydrogen-ion concentration of different soil suspensions was found to vary within wide limits, from a condition of high acidity to one of high alkalinity.

(4) Soils containing calcium in equilibrium with HCO_3^- and CO_2 have a very slightly alkaline reaction.

(5) The effect of heating, grinding, and of varying the ratio of soil to water on the hydrogen-ion concentration was studied.

(6) An electrometric method for the determination of the lime requirement of soils is suggested.

(7) The addition of sodium chlorid, potassium chlorid, and barium chlorid to certain soil suspensions was found to increase the hydrogen-ion concentration.

[1] For a review of the literature concerning the application of the hydrogen electrode to biochemistry the reader is referred to the following:

Schmidt, C. L. A. Changes in the H^+ and OH^- concentration which take place in the formation of certain protein compounds. *In* Jour. Biol. Chem., v. 25, no. 1, p. 63–79, 9 fig. 1916. Bibliographical footnotes.

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(8) Several phases of the phenomena designated "adsorption" were studied, with special reference to the removal of OH ions by the soil from solutions of various hydrates.

(9) There appears to be a simultaneous removal of positive and negative ions from solution by soils.

(10) Some general theoretical considerations with regard to the relation of adsorption to chemical reactions in soils are presented.

(11) A convenient method of procedure for utilizing the hydrogen electrode in soil studies is described.

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LIFE HISTORY OF HABROCYTUS MEDICAGINIS, A RECENTLY DESCRIBED PARASITE OF THE CHALCIS FLY IN ALFALFA SEED

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INTRODUCTION

The following account of *Habrocytus medicaginis* Gahan is the result of observations concerning its parasitism upon *Bruchophagus funebris* How. inhabiting alfalfa seed (*Medicago sativa*). The observations were begun in the fall of 1912 and continued into the year 1915. The field observations and collections extended over several of the States west of the Rocky Mountains. The laboratory studies were conducted at Glendale and Pasadena, Cal.

DISCOVERY OF THE PARASITE

This new hymenopterous parasite, *H. medicaginis*, was first found by the writer on September 28, 1912. Several specimens had emerged from alfalfa seeds infested by *B. funebris* collected at Yuma, Ariz., on August 30. Adults of the parasite continued to emerge from the seeds until October 15 of the same year.

The parasite was again reared on November 4, 1912, from infested alfalfa seeds taken at Chino, Cal., on September 24; from this lot of seeds individuals continued to emerge until July 12, 1913. Additional alfalfa seeds collected at Tulare, Cal., on October 1, 1912, yielded this species at different times between June 11, 1913, and April 9, 1914.

Alfalfa seeds dissected on March 6, 1913, at Glendale, Cal., showed larvæ of this species feeding upon the dead larvæ of *B. funebris*. Some of the parasite larvæ were reared to the adult stage as early as April 22.

The writer made many collections of alfalfa seeds throughout the different alfalfa seed-growing districts between the Rocky Mountains and the Pacific coast during the seasons of 1913 and 1914. From these seeds adults of *H. medicaginis* emerged as follows: Dos Palos, Cal., October 11, 1913; Stockton, Cal., June 16, 1914; Brawley, Cal., June

17, 1914; Red Bluff, Cal., September 23, 1914; Pendleton, Oreg., September 23, 1914; Twin Falls, Idaho, September 23, 1914; Blackfoot, Idaho, September 29, 1914; Gunnison, Utah, October 10, 1914; Bishop, Cal., April, 1915; Manti, Utah, June 5, 1915; Aberdeen, Idaho, June 16, 1915; and Nephi, Utah, June 16, 1915. The total rearings of this parasite brought the number of adults up to several hundred specimens from these different localities. Figure 1 shows the known distribution of the insect.

In recent years various members of the Bureau of Entomology, and others, have reared *B. funebris*, together with miscellaneous insects emerging from both clover and alfalfa seeds. These specimens were examined and it was found that *H. medicaginis* was reared from alfalfa seed pods infested by *B. funebris* as follows:

August 12, 1908, Mesilla Park, N. Mex., by C. N. Ainslie.
———— 1908, Chico, Cal., by R. McKee.
———— 1910, Sacramento, Cal., by O. E. Bremner.
September 24, 1910, Wellington, Kans., by E. O. G. Kelly.
———— 1910, Wellington, Kans., by T. H. Parks.
September 9, 1910, Wellington, Kans., by H. Osborn.
———— 1912, Cavite, S. Dak., S. Halvardgaard.
———— 1913, Newell, S. Dak., C. N. Ainslie.
———— 1914, Salt Lake City, Utah, by T. R. Chamberlin.

CLASSIFICATION AND DESCRIPTION

H. medicaginis belongs to the hymenopterous superfamily Chalcidoidea, family Pteromalidae, subfamily Pteromalinae. Specimens reared

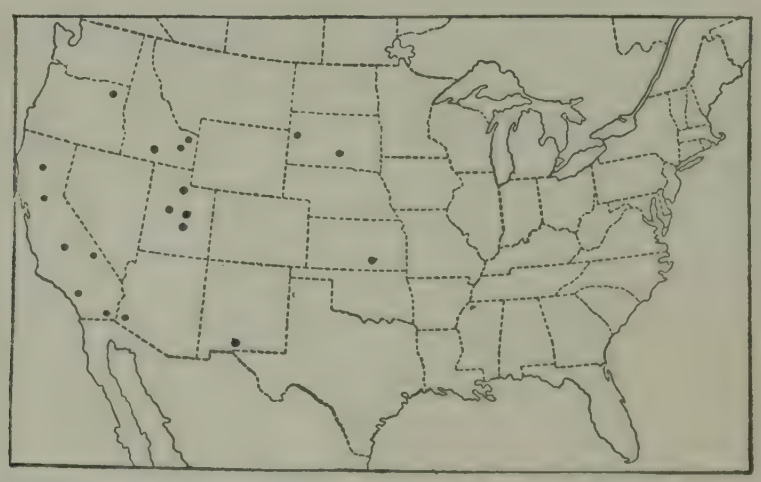


FIG. 1.—Map of the United States, showing the known distribution of *Habrocytus medicaginis*.

by the writer from *B. funebris* in alfalfa seeds at Yuma, Ariz., were described by Gahan as a new species.[1]

The description of the female is as follows:

Length about 1.7 mm. Head and thorax closely punctate, the punctures on the medial portion of the mesoscutum slightly larger than those on the scapulæ and scutellum; antennæ with two ring-joints; pedicel and first funicle joint, excluding

[1] Gahan, A. B. Descriptions of new genera and species, with notes on parasitica Hymenoptera. *In* Proc. U. S. Nat. Mus., v. 48, p. 163. 1915.

the ring-joints, about equal; following funicle joints a little longer than the first and a trifle longer than broad; viewed from in front the head is broader than long, the clypeal region with converging striæ and a deep median sinus on the anterior margin; viewed from above the head is slightly broader than the thorax, narrow anteroposteriorly, the occiput slightly concave, the ocellocular line longer than the lateral ocellar line, the lateral ocellar line not equal to half the postocellar line; pronotum strongly transverse with a sharp margin anteriorly; propodeum short, without a neck, with a median carina and lateral folds, the region between the lateral folds more or less distinctly wrinkled and with a fovea-like depression at the base and another at the apex of the fold; the region outside the lateral folds is usually more faintly sculptured with indistinct lines; propodeal spiracles elliptical; marginal and postmarginal veins subequal, the stigmal one-third shorter; abdomen conic-ovate, about as long as the head and thorax and nearly smooth, the dorsal segments beyond the first with very faint transverse lines. Head and thorax æneous; antennæ brown, the scape slightly paler beneath; wings hyaline; all coxæ æneous like the thorax, all trochanters and femora black with an æneous tinge; tibiæ and tarsi usually reddish yellow, the former often brownish except at apex; apical joint of all tarsi dark; abdomen polished aeneous.

LIFE HISTORY OF THE HOST

The host insect (*B. funebris*) of *H. medicaginis* completes its entire life development within the growing seeds of alfalfa, red clover (*Trifolium incarnatum*), and wild species of Medicago. After reaching maturity the adult eats a hole through the seed wall and through the wall of the seed pod to make its escape. *B. funebris* may pass through from one to four or five generations in a single season.

METHOD OF STUDYING THE PARASITE

The fact that *H. medicaginis* completed its entire development within the unbroken walls of an alfalfa seed made it necessary for the writer to dissect many seeds under a microscope and remove this parasite in its different stages for special study. Small parasite larvæ removed from seeds were placed singly upon a larva of their host. The host and parasite were then placed in a small cavity made in a 7-mm. cork and covered by a glass vial (Pl. 4, fig. B). A most satisfactory method of observing one of these larvæ in its development was to place it upon a larva of *B. funebris* and keep both host and its parasite in a cavity made between two layers of sheet cork. The upper layer of cork could then be removed to expose the parasite larva.

STAGES OF HOST SHOWING PARASITISM

H. medicaginis is parasitic upon the larval stage of its host with possibly a few exceptions. Microscopic dissections of many infested alfalfa seeds showed 77 larvæ of this species, each feeding externally upon the larval stage of its host (Pl. 4, fig. D). This parasite was in no case found to be attacking the pupa of *B. funebris*. Only a single parasite is able to develop upon its host within the walls of a single infested alfalfa seed.

The parasite larva in completing its development usually destroys the larva of its host with the exception of the head and mandibles. If two parasites chance to be upon a single host, one dies before development continues for any length of time.

APPEARANCE OF THE INSECT IN THE FIELDS

Throughout the Southwestern States the first adults make their appearance in the fields as early as March and April, simultaneously with the development of seed pods upon the earliest alfalfa plants. Out of nearly 100 hibernating larvæ kept under observation at the laboratory, 23 emerged as adults in March, 39 in April, 8 in May, and 4 in June. They attack the first generation of larvæ of *B. funebris* infesting the earliest isolated plants and increase throughout the summer in accordance with the abundance of their host insects.

OVIPOSITION

The adult female, frequently seen to be active on the blossoms and soft green seed pods in the alfalfa fields, is apparently able to locate the pods in which seeds have previously been infested by *B. funebris*. She selects her position upon the green pod directly over an infested seed. During oviposition the head is slightly elevated and the antennæ are held directly forward. The tip of the abdomen is lowered almost to the surface of the seed pod. The ovipositor is forced through the soft walls of the pod and into the watery seed. It is necessary for the egg of the parasite to be placed within the infested seed and upon the larva of its host in order that the newly hatched parasitic larva may secure food for its development.

THE LARVA

DEVELOPMENT

For several hours after emerging from the egg, the larva of the parasite may move about on its host without feeding, but when it once begins to attack its host and take food, its development follows rapidly. The writer's observations show that a growing larva of this species may completely destroy its host and become fully developed within a minimum period of five or six days after taking its first food.

DORMANT PERIOD

When the parasite larva has completed its development and consumed all of its available food, a period of rest frequently follows. The occurrence and duration of this resting period depends upon the moisture and temperature conditions to which the seed is subjected. A larva of *H. medicaginis* completing its growth within a moist seed of a green and growing pod will almost invariably transform to the pupal stage at once and emerge as an adult in due time, but if the infested alfalfa seed has

become dried from the hot desert winds before the parasitic larva has completed its development, a prolonged resting period may follow. This period may vary from a few weeks to a year. With combined moisture and a warm temperature the insect resumes its development toward the formation of the pupal stage.

DESCRIPTION

The larva is grublike and almost white in color and averages 1.6 mm. in length when fully developed. It is cylindrical in shape and rounded anteriorly and posteriorly. Head medium-sized and slightly bilobed. Mandibles almost invisible. It has a small inconspicuous tubercle on each eye lobe. There are 13 body segments of approximately equal length, except the first two, which are slightly longer; segmentation medium. Body skin usually slightly wrinkled, but sometimes smooth, glossy, and free from pubescence. Anal segment divided into a dorsal and a ventral lobe. Three fine setæ on dorsal lobe (Pl. 4, fig. C).

LENGTH OF LARVAL STAGE

The length of the larval stage depends greatly, as has been previously stated, upon the resting period following the development of the larva. A small newly hatched larva began feeding upon its host under observation on April 23. It showed noticeable growth from day to day, and by April 27 it had completely killed its host. On May 5 the larva transformed to the pupal stage. Another newly hatched larva dissected from a green alfalfa seed began feeding upon its host on September 5 and by September 7 the host was killed. The larva was apparently fully developed by September 11. Others removed from alfalfa seeds in August did not pupate until the following April. The minimum length of the larval stage as observed by the writer is normally about 12 days. The maximum length is a year or more.

PREPUPAL FORM

Just before entering the pupal stage the larva of *H. medicaginis* discharges an excessive amount of excrement. This is followed by a lengthening of the anterior body segments and the shaping of the pupa within the larval skin. The prepupal form requires two or three days unless retarded by unfavorable conditions.

THE PUPA

FORMATION

Pupation takes place after the pupal form has developed within the larval skin. The larval skin breaks along the dorso-anterior margin and is slowly worked back until the newly formed pupa is exposed.

DESCRIPTION

The newly formed pupa is white with salmon-colored eyes and ocelli; before transforming to the adult stage it becomes almost black in color. It averages 1.8 mm. in length. The head and tip of abdomen are bent slightly forward. The wing pads, legs, and antennæ, folded close to the body, are visible through the thin pupal skin (Pl. 4, fig. E).

LENGTH OF PUPAL STAGE

The length of the pupal stage varies greatly even in midsummer. This stage requires about 10 days under favorable field conditions in midsummer. Under laboratory conditions approximating out-of-door temperatures the pupal stage varied from 10 to 52 days, the long pupal stages being recorded in the months of March and April.

Larvæ, after hibernating through the winter, pupated in the spring and showed the following average period in the pupal stage: In March, 23 pupæ averaged 14 days; in April, 39 averaged 23 days; in May, 8 averaged 21 days; and in June, 4 averaged 18 days.

ADULT

The adult (Pl. 4, fig. A), upon emerging from the thin pupal skin, finds itself completely inclosed within the alfalfa seed and within the seed pod. It at once gnaws its way out, escaping by the small irregular opening which it makes.

CHOICE OF HOST PLANTS

H. medicaginis was not found to be present as a parasite of *B. funebris* when the latter infested the seed of red clover. This was true even where the red clover was taken near alfalfa fields and *H. medicaginis* was known to be present.

RELATIVE PROPORTION OF SEXES

Some localities from which this species was reared showed apparently no males, while in other localities a few males were found. The proportion of males is, however, very small to that of the females. Reared adults were counted to get the proportion of sexes. It was found that 270 of these were females and 9 were males. This showed a ratio of 1 male to 30 females.

SEASONAL HISTORY

In western Arizona and southern California *H. medicaginis* appears in the adult stage as early as the month of March. It is in its greatest abundance during July and August on irrigated alfalfa fields. On drier lands, where the seeds are subjected to desert conditions, many of the larvæ are driven to an early dormant period and the adults become less abundant in the hot months. Under extremely variable conditions there are from one to at least four generations in a single season. One larva

removed with its host from an alfalfa seed collected on December 19, 1913, transformed to the pupal stage in May, 1915, and emerged as an adult on June 2, 1915. On the other hand, a newly hatched larva of this species was placed upon its host on April 22, entered the pupal stage on May 5, and emerged as an adult on May 19. Another newly hatched larva placed under observation on September 5 had developed, pupated, and emerged as an adult by September 24. Infested alfalfa seeds which were collected on October 1, 1912, showed an adult of *H. medicaginis* emerging as late as April 9, 1914. These observations show that a period of from about 30 days to 1 year, and almost 2 years in exceptional cases, may be required for the completion of a single generation.

HIBERNATION

H. medicaginis hibernates in the larval stage within the infested alfalfa seeds which remain on the standing alfalfa, or on the ground when winter approaches. The undeveloped larvæ and those still in the pupal stage are usually killed by the first severe frost. In the mild climate of southern California occasional individuals of this species hibernate in the pupal stage. Nearly 100 larvæ of *H. medicaginis* were removed from their natural inclosure within the alfalfa seeds and placed in cavities between two layers of sheet cork. Of these larvæ 74 lived throughout the winter, entering the pupal stage in the months of March, April, and May at Glendale, Cal.

RATE OF PARASITISM

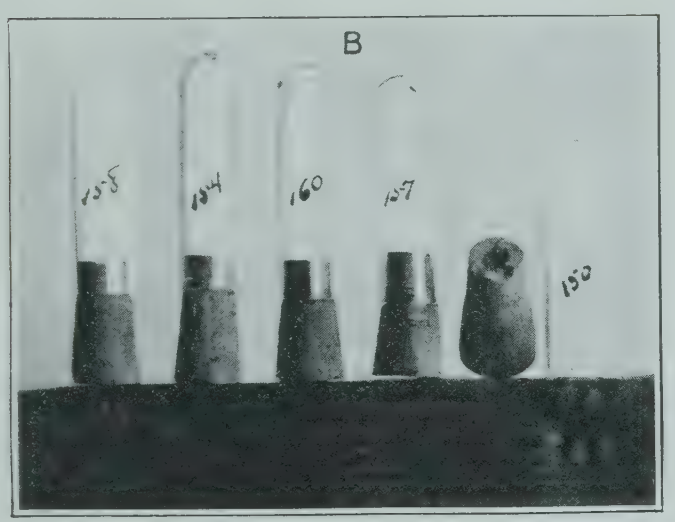
While this species is generally distributed throughout the alfalfa seed-growing districts of the United States, the rate of parasitism is not so large as might be expected. The comparative rearings of *H. medicaginis* and their host (*B. funebris*) show parasitism by *H. medicaginis* in several localities to be about as follows: Corcoran, Cal., 0.8 per cent; Tulare, Cal., 2.8 per cent; Chino, Cal., 2.8 per cent; and Yuma, Ariz., 4.9 per cent.

PLATE 4

Habrocytus medicaginis:

Fig. A.—Adult.
Fig. B.—Cages for rearing parasite larvæ.
Fig. C.—Larva.
Fig. D.—Larva destroying its host larva.
Fig. E.—Pupa.

A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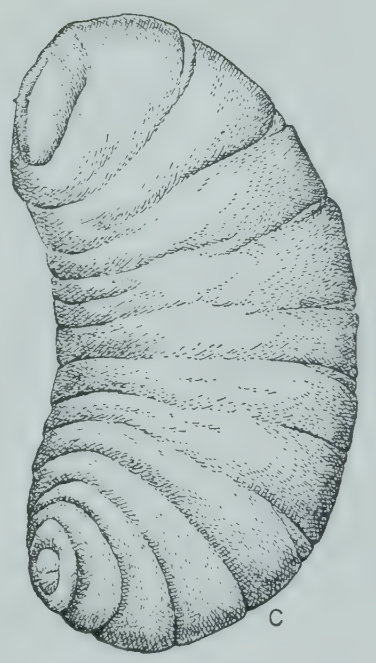
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DAILY TRANSPIRATION DURING THE NORMAL GROWTH PERIOD AND ITS CORRELATION WITH THE WEATHER

By LYMAN J. BRIGGS, *Biophysicist in Charge, Biophysical Investigations*, and H. L. SHANTZ, *Plant Physiologist, Alkali and Drought Resistant Plant Investigations, Bureau of Plant Industry*

This paper deals with the daily transpiration of a part of the crop plants included in the water-requirement experiments at Akron, Colo., in 1914 and 1915.[1] The principal objects of the measurements were the determination of (1) the march of transpiration during the growth period, and (2) the extent to which the daily transpiration is correlated with various weather factors.

EXPERIMENTAL METHODS

The plants were grown in galvanized-iron pots, containing about 115 kgm. of soil and provided with tight-fitting covers with openings for the stems of the plants. The annular space between the cover and the stem of each plant was sealed with a plastic wax. Direct evaporation from the soil was thus avoided and the loss of water limited to transpiration.[2]

Twenty-two crops were included in the daily weighings in 1914 and 23 crops in 1915. Each crop was represented by six pots of plants (Pl. 5) weighed independently. The weighings were made with an accuracy of 0.1 kgm. by means of a spring balance checked before and after each series of weighings against a standard weight of 130 kgm. The balance and weighing device are shown in Plate 6. The daily weights served also as a basis for determining the quantity of water to be added daily to each pot to insure an adequate water supply.

In differentiating between the transpiration of consecutive days it is desirable that the weighings be made at a time when the plants are losing very little water. Automatic records show that the transpiration is at a minimum just before sunrise.[3] The daily weighings, which required the time of three men for an hour, were accordingly begun in the morning as soon as there was light enough to work and completed before the transpiration response to sunlight had set in.

[1] Acknowledgment is gratefully made of the efficient and valued assistance of Messrs. R. L. Piemeisel, F. A. Cajori, P. N. Peter, J. D. Hird, G. Crawford, R. D. Rands, A. McG. Peter, H. W. Markward, H. Shattyn, and T. R. Henault at Akron in 1914 and 1915. Mr. W. H. Heal has also aided very materially in the reduction of the measurements.

[2] Briggs, L. J., and Shantz, H. L. The water requirement of plants. I.—Investigations in the Great Plains in 1910 and 1911. U. S. Dept. Agr. Bur. Plant Indus. Bul. 284, p. 9. 1913.

[3] ——— ——— Hourly transpiration rate on clear days as determined by cyclic environmental factors. *In* Jour. Agr. Research, v. 5, no. 14, p. 583–650, 22 fig., pl. 53–55. 1916. Literature cited, p. 648–649.

The transpiration of the plants in each pot,as determined by the weighings, was plotted daily as a check on the weighing and watering records. The daily transpiration of the first five crops of the 1914 series, traced directly from the original graphs, is shown in figure 1. These graphs are typical of the series and show the proportional response of the individual pots of plants to the fluctuations in weather factors. The daily transpiration of each crop is represented by the mean value of the six individual determinations, which minimizes slight errors in the weights of the individual pots, and abnormalities in the transpiration rate of individual plants. Inspection of figure 1 will show the close agreement of the individual determinations. While the plants in some pots of a given series transpire more than others, owing to differences in stand or size of the plants, the daily fluctuations are very nearly proportional.

The weather factors measured included solar radiation, air temperature, wet-bulb depression, and wind velocity. These factors, as well as evaporation, were integrated for each day. The solar radiation and the wet-bulb depression were measured by differential thermographs and the air temperature by a standardized air thermograph. The wind velocity was recorded by an anemometer located 3 feet above the ground. The evaporation was measured by means of a shallow blackened tank 6,540 sq. cm. in area, exposed at the level of the plants, and also by means of a large tank 8 feet in diameter and 2 feet deep, with the water surface at the ground level.[1]

DAILY TRANSPIRATION AND THE DAILY INTENSITY OF THE WEATHER FACTORS DURING THE GROWTH PERIOD

MEASUREMENTS IN 1914

The daily transpiration of 22 crops grown in 1914 is given in Table I, the daily loss being expressed in kilograms per pot. The small grains were well established before the daily weighings were begun, and had lost during the previous month approximately 10 per cent of the total water transpired during the entire growth period. The daily weighings in the case of the other crops cover the entire growth period after the daily loss had reached one-tenth of a kilogram or more per pot.

[1] For a further description of the methods and apparatus employed, see Briggs and Shantz, op. cit., 1916, p. 584–585, 625; and Briggs, L. J., and Belz, J. O. Dry farming in relation to rainfall and evaporation. U. S. Dept. Agr. Bur. Plant Indus. Bul. 188, p. 17. 1910.

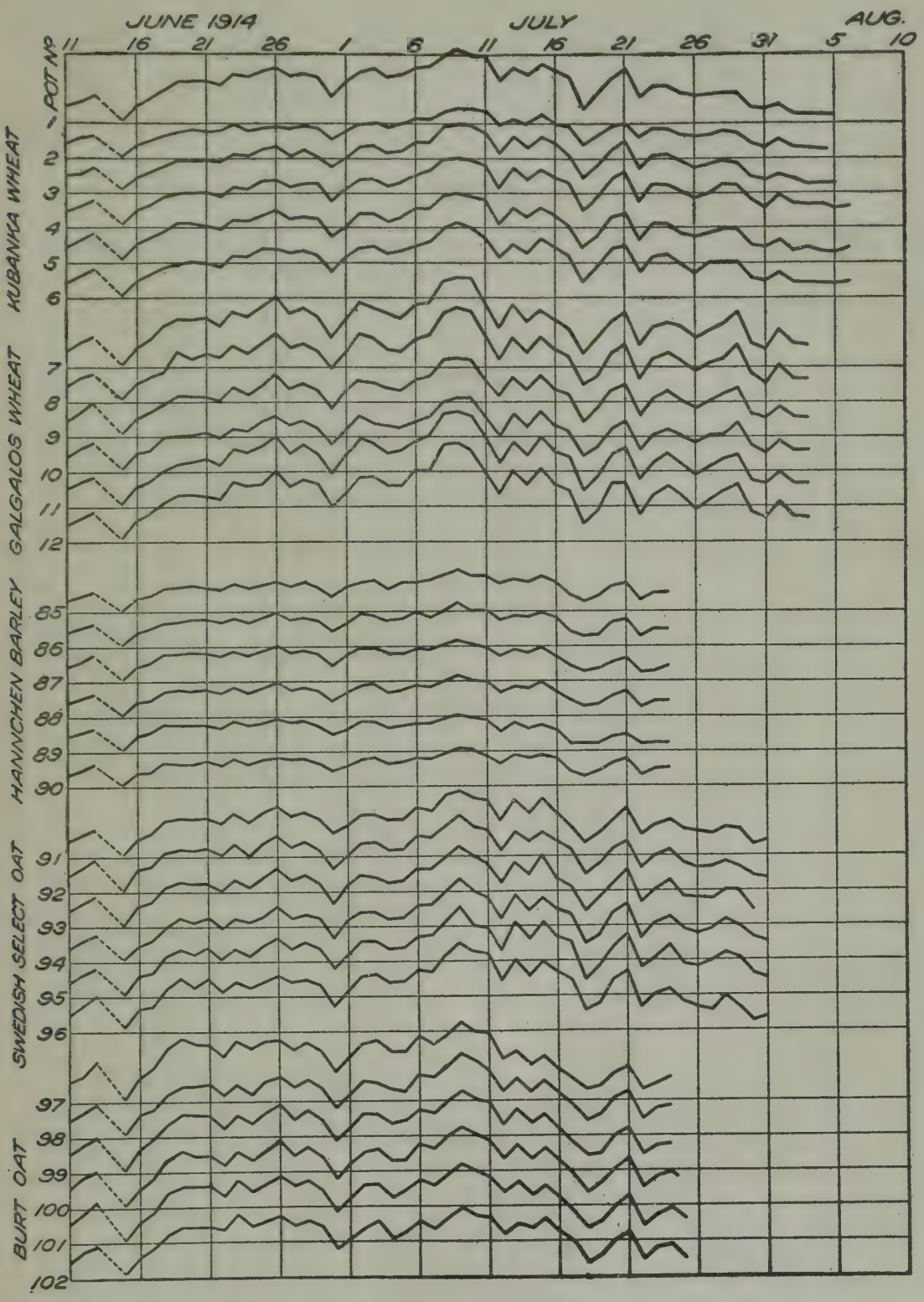

FIG. 1.—Graphs showing the daily transpiration from the individual pots of plants which constituted the first five sets in the transpiration measurements in 1914.

TABLE I.—*Measurements of environmental factors and daily transpiration, June 11 to September 25, 1914*

JUNE 11 TO 30

Item.	Pot No.	June.																			
		11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Evaporation (shallow tank), kgm. per sq. meter							6.76	8.81	11.86	13.15	10.86	12.20	10.58	12.17	10.79	12.05	17.67	16.65	12.84	12.14	5.54
Evaporation (deep tank) mm		3.10	5.08	6.40	3.36	2.37	4.65	4.90	5.87	7.39	7.57	8.46	7.47	6.15	6.71	8.69	13.75	5.87	8.36	8.18	3.33
Evaporation (deep tank) inches		.122	.200	.252	.132	.092	.183	.193	.231	.291	.298	.333	.294	.242	.264	.342	.541	.231	.329	.322	.131
Air temperature, max °C		26.1	30.6	32.2	24.4	16.1	22.2	26.7	31.7	32.8	30.6	32.2	26.1	33.9	31.7	33.9	29.4	29.4	33.3	28.3	21.1
Air temperature, min °C		10.6	8.9	12.8	11.7	9.4	11.7	12.8	11.7	14.4	11.7	12.8	13.3	13.3	14.4	13.9	8.3	7.2	7.8	6.7	7.8
Air temperature integrated, mean °C		19.4	20.0	23.3	17.8	13.3	17.2	20.0	23.3	23.9	21.7	22.8	20.0	23.9	22.8	26.1	20.0	19.4	21.1	20.0	15.0
Air temperature, max °F		79	87	90	76	61	72	80	89	91	87	90	79	93	89	93	85	85	92	83	70
Air temperature, min °F		51	48	55	53	49	53	55	53	58	53	55	56	56	58	57	47	45	46	44	46
Air temperature integrated, mean °F		67	68	74	64	56	63	68	74	75	71	73	68	75	73	79	68	67	70	68	59
Integrated radiation, cal. per sq. cm.									959	974	890	934	984	1,011	1,032	836	1,069	1,006	890	1,006	553
Integrated wet-bulb depression, deg. hrs.									178	182	167	173	145	187	118	176	264	203	212	168	84
Wind velocity miles per hr.		8.0	7.7	1.3	6.9	3.8	6.0	3.8	2.3	7.5	5.1	6.4	5.8	4.4	5.2	6.3	12.7	3.7	5.3	8.3	7.1
Wind velocity meters per sec.		3.6	3.4	.6	3.1	1.7	2.7	1.7	1.0	3.4	2.3	2.9	2.6	2.0	2.3	2.8	5.7	1.7	2.4	3.7	3.2
Daily transpiration, kgm.:																					
Wheat, Kubanka	1–6	1.2	1.5	2.0	.....	.2	*1.1	1.6	2.1	2.5	2.6	2.5	2.3	3.0	2.8	3.3	3.4	3.0	3.2	3.0	1.8
Wheat, Galgalos	7–12	1.2	1.8	2.2	.....	.2	1.4	1,8	2.6	3.1	3.1	3.2	2.9	3.9	3.5	4.0	4.8	3.7	4.2	3.7	2.3
Oat, Swedish Select	91–96	1.1	1.6	2.2	.....	.2	1.4	1.7	2.8	3.3	3.0	3.2	2.6	3.3	2.8	3.5	4.0	3.2	3.6	3.1	1.8
Oat, Burt	97–102	1.3	2.0	2.6	.....	.2	1.5	2.2	3.3	3.9	3.8	3.8	3.1	4.1	3.5	4.1	4.5	3.7	4.2	3.6	2.1
Barley, Hannchen	85–90	1.0	1.3	1.7	.....	.2	1.0	1.2	1.8	1.8	1.9	1.9	1.7	2.1	1.7	2.0	2.3	2.0	2.1	1.8	1.1
Rye, spring	103–108	1.3	1.7	2.2	.....	.2	1.3	1.9	2.7	3.1	3.0	3.0	2.6	3.5	2.8	3.2	3.4	2.8	3.0	2.6	1.5
Cowpea	169–174	.1	.4	.6	.....	.1	.3	.5	.9	1.3	1.5	1.3	1.3	1.9	1.7	2.3	2.8	2.0	2.2	2.1	1.3
Lupine	175–180	.1	.4	.4	.....	.1	.2	.4	.7	.9	1.0	.7	.7	1.1	.9	1.2	1.3	1.2	1.3	1.2	.8
Millet, Kursk	181–186	.....	.2	.2	.....	.1	.0	.2	.5	.7	.9	.8	.8	1.4	1.2	1.6	2.0	1.7	1.9	1.6	.9
Millet, Siberian	187–192	.....	.2	.2	.....	.1	.0	.2	.5	.7	.9	.8	.8	1.5	1.2	1.8	2.4	1.9	2.0	1.7	1.0
Corn, Northwestern Dent	217–222	.2	.1	.5	.....	.1	.1	.3	.6	.9	1.0	.9	.8	1.5	1.3	1.6	2.1	1.7	2.0	1.7	.8
Corn, Algeria	238–242	.1	.0	.3	.....	.1	.0	.0	.2	.4	.5	.3	.3	.7	.5	.8	1.0	.9	1.0	.9	.5
Sorghum, Minnesota Amber	205–210	.0	.0	.1	.....	.1	.....	.0	.2	.3	.4	.2	.2	.6	.5	.7	1.0	.9	1.0	1.0	.5
Sorghum, Dakota Amber	211–216	.0	.0	.2	.....	.1	.....	.1	.2	.3	.4	.2	.3	.6	.6	.8	1.0	1.0	1.1	1.0	.6
Sudan grass	410–414	.1	.0	.2	.....	.1	.1	.0	.1	.2	.3	.0	.1	.3	.2	.3	.6	.6	.7	.6	.3
Sudan grass (in open)	319–323	.0	.0	.0	.....	.0	.0	.0	.0	.0	.2	.0	.0	.2	.0	.2	.2	.2	.3	.2	.2
Amaranthus	163–168	.2	.6	.8	.....	.1	.5	.8	1.3	1.5	1.5	1.3	1.1	1.5	1.2	1.4	1.5	1.3	1.4	1.1	.6
Alfalfa, E23–20–52	109–114	.0	.2	.2	.....	.1	.2	.3	.5	.7	.9	.8	.8	1.2	1.1	1.5	1.7	1.5	1.6	1.7	1.1
Alfalfa, E23	115–120	.0	.3	.3	.....	.1	.2	.4	.6	.8	1.0	1.0	.9	1.4	1.3	1.8	2.1	1.8	2.0	2.0	1.4
Alfalfa, E23 (in open)	295–300	.2	.2	.3	.....	.0	.2	.3	.6	.8	.9	.9	.8	1.4	1.2	1.6	1.8	1.6	1.8	1.6	1.2
Alfalfa, 162–98A	127–132	1.2	1.8	2.3	.....	.3	1.4	2.3	3.2	3.7	3.9	4.0	3.6	4.5	4.3	5.1	6.5	5.2	5.6	4.9	3.8

Item.	Pot No.	July.																				
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Evaporation (shallow tank), kgm. per sq. meter		9.27	12.84	12.82	9.08	10.40	13.43	11.28	13.92	14.34	15.04	12.26	7.89	11.86	9.66	14.06	12.87	10.82	5.08	6.18	10.70	13.94	5.69
Evaporation (deep tank), mm		6.35	6.73	7.36	5.44	6.71	8.81	8.03	9.45	10.77	10.62	8.11	4.83	6.23	7.06	9.78	9.32	9.66	3.68	4.45	6.71	10.53	4.34
Evaporation.....inches		.250	.265	.290	.214	.264	.347	.316	.372	.424	.418	.319	.190	.245	.278	.385	.367	.380	.145	.175	.264	.414	.171
Air temperature, max. °C		25.6	28.9	30.0	28.9	31.1	31.1	30.6	34.4	33.3	33.9	33.9	33.9	33.3	32.8	35.0	27.2	27.2	22.2	25.0	31.1	32.2	22.2
Air temperature, min. °C		12.2	11.7	9.4	12.8	13.3	13.3	12.8	13.3	15.0	15.6	17.8	13.9	15.0	17.8	18.3	16.7	13.9	13.3	15.0	17.2	12.8	16.1
Air temperature, integrated mean.....°C.		18.9	20.6	20.6	21.1	22.2	21.1	21.1	23.9	23.9	25.0	26.1	22.8	25.0	24.4	26.1	21.1	19.4	17.2	20.0	23.9	22.2	17.8
Air temperature, max., °F.		78	84	86	84	88	88	87	94	92	93	93	93	92	91	95	81	81	72	77	88	90	72
Air temperature, min., °F.		54	53	49	55	56	56	55	56	59	60	64	57	59	64	65	62	57	56	59	63	55	61
Air temperature, integrated, mean.....°F.		66	69	69	70	72	70	70	75	75	77	79	73	77	76	79	70	67	63	68	75	72	64
Integrated radiation, cal. per sq. cm		875	914	974	822	860	929	939	974	1,008	932	951	613	879	743	890	774	754	605	719	979	875	716
Integrated wet-bulb depression.....deg. hr.		138	170	176	111	162	173	145	228	248	216	231	153	181	174	240	199	158	44	68	148	201	66
Wind velocity, miles per hr		6.3	8.0	5.3	7.6	4.2	7.6	5.9	6.3	7.4	8.3	4.6	3.3	4.8	6.2	6.7	8.2	9.6	6.5	2.8	5.4	10.1	4.2
Wind velocity, meters per sec		2.8	3.6	2.4	3.4	1.9	3.4	2.6	2.8	3.3	3.7	2.1	1.5	2.1	2.8	3.0	3.7	4.3	2.9	1.3	2.4	4.5	1.9
Daily transpiration, kgm.:																							
Wheat, Kubanka	1–6	2.7	3.4	3.5	3.0	3.2	3.7	3.8	4.6	5.0	4.8	4.4	2.9	3.9	3.2	4.0	3.4	2.8	1.0	1.9	3.1	3.5	1.7
Wheat, Galgalos	7–12	3.4	4.5	4.3	3.7	3.6	4.4	4.7	6.2	6.4	6.1	4.6	3.0	4.5	3.4	4.5	3.5	3.1	1.0	1.8	3.4	3.9	1.6
Oat, Swedish Select	91–96	2.8	3.6	3.6	3.0	3.1	4.0	3.9	4.9	5.7	4.9	4.6	3.0	4.6	3.6	4.6	3.6	3.0	1.0	1.8	3.1	3.9	1.6
Oat, Burt	97–102	3.0	3.9	4.0	3.2	3.3	4.3	3.9	4.7	5.5	4.9	4.6	3.1	4.0	3.1	3.8	2.9	2.0	.8	1.3	2.4	3.0	1.0
Barley, Hannchen	85–90	1.7	2.2	2.2	1.7	1.9	2.2	2.1	2.5	2.8	2.5	2.4	1.7	2.1	1.9	2.2	1.8	.9	.5	.7	1.4	1.6	.6
Rye, spring	103–108	2.0	2.8	2.8	2.0	2.4	2.8	2.6	3.3	3.6	3.3	3.3	2.4	3.0	2.7	3.5	2.8	2.0	.8	1.3	2.3	2.9	1.1
Cowpea	169–174	1.4	1.8	2.3	2.1	2.5	3.2	2.7	3.3	3.7	3.5	3.5	2.3	3.5	2.7	3.8	2.7	1.9	.9	1.3	2.5	3.1	1.5
Lupine	175–180	1.1	1.5	1.4	1.3	1.4	1.6	1.4	1.6	2.1	1.8	1.9	1.3	1.9	1.6	2.0	1.6	1.2	.7	1.0	1.6	2.0	.9
Millet, Kursk	181–186	1.2	1.8	2.0	1.7	1.9	2.3	1.9	2.5	3.0	2.6	2.7	1.8	2.7	2.1	2.8	1.8	1.1	.5	.9	1.8	2.2	.7
Millet, Siberian	187–192	1.4	1.9	2.0	1.9	2.0	2.1	2.0	2.6	3.1	2.8	3.1	1.9	2.9	2.3	3.0	2.0	1.3	.8	1.1	2.1	2.7	.8
Corn, Northwestern Dent	217–222	1.4	1.9	2.1	1.8	2.2	2.5	2.3	3.1	3.7	3.1	3.1	2.0	2.9	2.2	3.2	1.9	1.3	.6	1.1	2.1	2.6	.8
Corn, Algeria	238–242	.8	1.2	1.3	1.5	1.7	2.2	1.9	2.7	3.3	2.9	3.1	1.9	3.2	2.4	3.2	2.3	1.8	.6	1.4	2.7	3.3	1.0
Sorghum, Minnesota Amber	205–210	.8	1.4	1.5	1.4	1.8	2.1	2.1	2.9	3.4	2.9	3.0	1.9	3.0	2.4	3.1	2.1	1.9	.6	1.3	2.3	2.9	1.0
Sorghum, Dakota Amber	211–216	1.0	1.5	1.6	1.5	1.8	2.2	1.9	2.9	3.4	2.7	3.0	1.7	2.9	2.2	3.3	2.2	1.8	.9	1.1	2.6	2.9	1.1

TABLE I.—*Measurements of environmental factors and daily transpiration, June 11 to September 15, 1914*—Continued

JULY 1 TO 22—continued

Item.	Pot No	July.																					
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Daily transpiration, kgm.—Continued.																							
Sudan grass	410–414	.6	1.0	1.2	1.3	1.6	2.0	1.9	2.7	3.5	3.2	3.3	2.1	3.3	2.6	3.8	2.6	2.4	.8	1.4	2.8	3.7	1.1
Sudan grass (in open)	319–323	.4	.4	.7	.5	.9	1.3	1.2	1.8	2.3	2.3	2.6	1.7	2.5	2.3	3.1	2.1	2.0	.5	1.1	2.4	3.0	.9
Amaranthus	163–168	.9	1.1	1.1	1.1	1.5	1.6	1.5	1.6	2.1	1.5	1.6	1.1	1.6	C	.1	0	.....	.2	0	0	.3	.1
Alfalfa, E23–20–52	109–114	1.5	2.0	2.2	1.9	2.3	3.1	2.9	3.7	4.3	3.8	C	.1	.3	.2	.3	.3	.....	.4	.3	.7	1.1	.4
Alfalfa, E23	115–120	1.8	2.5	2.6	2.3	2.8	3.7	3.3	4.5	4.8	4.3	C	.1	.3	.1	.3	.2	.....	.4	.3	.7	1.2	.5
Alfalfa, E23 (in open)	295–300	1.7	1.9	2.3	1.9	2.5	3.1	3.0	4.3	4.6	4.6	C	.1	.2	.3	.4	.3	.0	.2	.3	.9	1.4	.6
Alfalfa, 162–98A	127–132	4.7	6.2	6.9	5.5	6.2	7.3	6.7	8.3	6.8	5.4	C	.3	.6	.6	1.0	1.0	.6	.6	.8	1.7	2.6	1.3

JULY 23 TO AUG. 13.

Item.	Pot No.	July.									August.												
		23	24	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10	11	12	13
Evaporation (shallow tank) kgm. per sq. meter		8.84	10.33	9.73	6.70	8.35	11.96	12.75	9.97	7.58	12.99	10.79	9.82	11.89	10.76	14.49	12.90	13.27	11.59	14.34	10.00	12.90	10.79
Evaporation (deep tank), mm		5.34	6.46	6.46	4.75	5.01	7.80	8.76	4.90	5.44	9.07	7.14	7.14	8.30	6.43	10.42	8.26	8.26	8.44	8.76	6.71	8.10	8.10
Evaporation (deep tank), inches		.210	.254	.254	.187	.197	.307	.345	.193	.214	.357	.281	.281	.327	.253	.410	.325	.325	.332	.345	.264	.319	.319
Air temperature, max., °C		31.1	32.2	32.2	33.3	35.6	33.9	35.0	31.1	31.1	33.3	29.4	29.4	33.9	33.3	35.0	35.0	35.0	27.8	27.2	27.8	34.4	32.2
Air temperature, min., °C		14.4	12.8	14.4	15.6	14.4	13.9	17.2	16.1	16.1	17.8	12.8	15.0	13.9	17.2	18.3	15.6	18.3	10.6	11.1	13.9	13.9	15.0
Air temperature, integrated, mean, °C		22.2	23.3	22.8	22.2	22.8	22.8	25.6	22.2	21.1	24.4	20.6	22.2	23.9	25.0	26.1	25.6	26.1	20.0	20.0	20.0	23.9	21.7
Air temperature, max., °F		88	90	90	92	96	93	95	88	80	92	85	85	93	92	95	95	95	82	81	82	94	90
Air temperature, min., °F		58	55	58	60	58	57	63	61	61	64	55	59	57	63	65	60	65	51	52	57	57	59

Air temperature, integrated, mean, °F.	.....	72	74	73	72	73	73	78	72	70	76	69	72	75	77	79	78	79	68	68	68	71
Integrated radiation, cal. per sq. cm.	.....	907	803	711	731	696	848	874	840	774	909	867	867	833	806	872	813	808	702	838	961	828
Integrated wet-bulb depression, deg. hrs.	.....	136	189	141	124	164	170	211	108	79	186	151	161	199	228	269	244	275	202	203	168	159
Wind velocity, miles per hour	.....	3.4	3.8	4.0	3.5	2.8	4.5	7.4	6.0	4.8	7.8	4.3	4.3	6.4	6.4	11.1	5.8	5.9	6.6	12.5	6.0	7.0	8.3
Wind velocity, meters per second	.....	1.5	1.7	1.8	1.6	1.3	2.0	3.3	2.7	2.1	3.5	1.9	1.9	2.9	2.9	5.0	2.6	2.6	2.9	5.6	2.7	3.1	3.7
Daily transpiration, kgm.																							
Wheat, Kubanka	1–6	2.6	2.7	2.1	1.7	2.0	2.3	2.2	1.2	.9	1.5	.9	.8	.8	.7	.....	.....	.....	.9	.....	.8	.....	.....
Wheat, Galgalos	7–12	2.9	3.4	2.8	2.1	2.6	3.1	3.7	1.7	1.3	2.4	1.5	1.4	.....	.....	.....	.....	.....	1.0	.....	.8	.....	.....
Oat, Swedish Select	91–96	2.5	3.0	2.0	1.7	1.8	2.4	2.0	1.0	1.0	.....	.....	.....	.....	.....	.....	.....	.....	1.3	1.4	.9	.....	.....
Oat, Burt	97–102	1.7	2.1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1.4	1.4	1.2	.....	.....
Barley, Hannchen	85–90	.9	1.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Rye, spring	103–108	1.9	2.1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Cowpea	169–174	1.9	2.4	2.2	1.6	1.8	2.3	2.3	.9	1.0	1.5	1.0	1.0	1.1	1.1	1.2	1.2	1.2	.9	.9	.8	.....	.....
Lupine	175–180	1.4	1.6	1.2	1.3	1.3	1.8	2.0	1.2	1.2	1.8	1.4	1.3	1.4	1.5	1.5	1.4	1.2	1.0	.9	.8	.....	.....
Millet, Kursk	181–186	1.5	1.9	1.4	1.2	1.5	1.8	2.1	.5	1.1	1.9	1.3	1.4	1.7	1.6	1.7	1.8	1.8	1.3	1.4	1.2	.....	.....
Millet, Siberian	187–192	1.7	2.2	1.6	1.5	1.7	2.2	2.3	.6	1.2	2.0	1.4	1.5	1.9	1.8	2.0	2.1	1.9	1.3	1.4	1.2	.....	.....
Corn, Northwestern Dent	217–222	1.7	2.2	1.8	1.4	1.8	2.0	2.6	.7	1.2	2.0	1.3	1.5	1.8	1.5	1.8	1.8	1.8	1.0	1.1	.8	1.5	1.1
Corn, Algeria	238–242	2.4	2.6	2.4	2.0	2.1	3.3	3.5	1.8	1.5	3.2	2.4	2.7	2.9	2.8	3.7	3.1	3.3	2.0	2.2	1.9	3.0	2.1
Sorghum, Minnesota Amber	205–210	1.9	2.6	2.1	1.9	2.0	2.7	3.3	1.9	1.4	2.6	1.9	2.0	2.6	2.4	3.2	2.7	2.9	1.8	2.2	1.7	2.5	1.8
Sorghum, Dakota Amber	211–216	1.9	2.5	2.2	1.8	2.0	2.7	3.2	1.7	1.3	2.4	1.8	2.0	2.4	2.2	2.9	2.6	2.7	1.6	1.9	1.5	2.4	1.5
Sudan grass	410–414	2.3	3.0	2.1	2.0	2.2	3.2	3.6	2.1	1.6	3.1	2.1	2.4	3.1	2.9	3.6	3.4	3.6	1.8	2.3	1.6	C	.2
Sudan grass (in open)	319–323	2.1	2.7	2.1	2.1	2.1	3.2	3.7	2.0	1.2	3.0	1.8	2.1	2.7	2.7	3.2	3.3	3.4	1.8	1.8	1.6	C	C
Amaranthus	163–168	.1	.3	.2	.3	.4	.6	.9	.3	.6	.9	.8	.8	.9	.9	1.0	1.0	1.1	1.0	1.0	.9	1.2	.3
Alfalfa, E23–20–52	109–114	.9	1.3	1.2	1.0	1.2	1.9	2.2	1.3	1.3	2.3	2.1	2.3	2.6	2.8	3.3	3.3	3.2	2.8	3.2	2.7	3.9	3.0
Alfalfa, E23	115–120	1.0	1.4	1.3	1.1	1.4	2.1	2.5	1.6	1.5	2.6	2.3	2.6	3.1	3.1	3.8	3.9	3.9	3.3	3.9	3.3	4.7	3.5
Alfalfa, E23 (in open)	295–300	1.2	1.7	1.6	1.4	1.7	2.5	2.9	2.0	1.4	3.1	2.5	2.6	3.0	3.2	3.6	4.0	4.1	3.4	3.3	2.9	4.3	3.2
Alfalfa, 162–98A	127–132	2.1	3.0	2.6	2.4	2.7	4.2	4.8	3.4	2.8	4.8	4.4	4.7	5.8	5.5	6.6	5.7	5.6	3.9	5.5	5.2	7.5	4.6

TABLE I.—*Measurements of environmental factors and daily transpiration, June 11 to September 25, 1914*—Continued

AUG. 14 TO SEPT. 4

Item.	Pot No.	August.																		September.			
		14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4
Evaporaton (shallow tank), kgm. per sq. meter		11.83	15.76	14.86	12.38	11.13	9.33	8.78	9.30	7.89	10.22	12.78	5.38	7.74	6.64	8.69	8.38	8.20	10.57	9.30	9.76	10.12	11.22
Evaporation (deep tank), mm		5.92	10.42	8.18	5.28	6.71	5.11	5.48	5.84	6.30	7.12	7.12	5.00	4.60	3.76	5.54	5.29	4.98	4.73	7.60	7.04	5.24	5.94
Evaporation (deep tank), inches		.233	.410	.322	.208	.264	.201	.216	.230	.248	.280	.280	.197	.181	.148	.218	.208	.196	.186	.299	.277	.226	.234
Air temperature, max., °C		34.4	36.7	37.8	38.3	30.6	31.1	33.3	28.9	30.0	28.9	31.7	21.7	27.8	22.2	28.3	28.9	30.6	29.4	28.3	27.8	32.2	33.3
Air temperature, min., °C		16.7	14.4	15.6	15.0	13.9	13.3	13.3	15.0	7.8	11.7	10.6	9.4	7.2	10.0	4.4	7.2	5.6	9.4	4.4	10.6	10.0	13.9
Air temperature, integrated mean °C		24.4	26.7	26.1	24.4	21.1	21.7	21.1	21.1	20.0	20.6	21.1	15.0	17.2	16.1	19.4	18.3	20.0	20.0	14.4	17.8	20.6	22.2
Air temperature, max., °F		94	98	100	101	87	88	92	84	86	84	89	71	82	72	83	84	87	85	83	82	90	92
Air temperature, min., °F		62	58	60	59	57	56	56	59	46	53	51	49	45	50	40	45	42	49	40	51	50	57
Air temperature, integrated mean, °F		76	80	79	76	70	71	70	70	68	69	70	59	63	61	67	65	68	68	58	64	69	72
Integrated radiation, cal. per sq. cm		889	810	900	699	682	795	657	674	502	843	837	556	435	615	731	783	680	810	727	743	810	606
Integrated wet-bulb depression, deg. hr		244	319	326	237	143	114	100	110	151	153	193	50	60	56	133	137	141	178	100	161	177	219
Wind velocity, miles per hr		6.0	7.1	5.1	5.9	7.1	6.9	4.2	7.3	5.9	6.8	8.5	9.0	8.1	4.3	4.3	4.4	4.4	4.8	7.8	7.3	4.4	4.8
Wind velocity, meters per sec		2.7	3.2	2.3	2.6	3.2	3.1	1.9	3.3	2.6	3.0	3.8	4.0	3.6	1.9	1.9	2.0	2.0	2.1	3.5	3.3	2.0	2.1
Daily transpiration, kgms:																							
Corn, Northwestern Dent	217–222	1.3	1.7	1.5	1.3	.1	.6	1.0	.5	.6	.6	.5	0	0	.6	.3	.4	.3	.....	.....	.....	.....	.....
Corn, Algeria	238–242	2.0	3.1	3.2	2.4	1.1	1.3	1.7	1.1	1.3	1.3	1.2	0	.2	.9	.9	1.1	.9	.....	.....	.....	.....	.....
Sorghum, Minnesota Amber	205–210	2.1	2.9	2.9	2.4	1.1	1.3	1.6	.9	1.3	1.4	1.6	.4	.2	.7	1.3	1.1	1.1	1.3	.....	.....	.....	.....
Sorghum, Dakota Amber	211–216	2.1	2.8	2.7	2.3	.9	1.3	1.4	.8	1.2	1.2	1.5	.3	0	.8	1.1	1.1	1.0	1.1	.....	.....	.....	.....
Sudan grass	410–414	.2	.4	.3	.5	.....	.4	.8	.5	.5	.6	.8	.1	.....	.7	.6	.7	.6	.8	.3	.6	.7	.8
Sudan grass (in open)	319–323	.2	.3	.4	.4	0	.5	.6	.4	.6	.7	.9	.3	.1	.5	.7	.7	.7	1.0	.6	.7	.9	1.0
Amaranthus	163–168	0	.2	0	0	.....	.1	.4	0	.2	.2	.1	.....	.....	.4	.2	.4	.2	.3	.1	.3	.4	.4
Alfalfa, E23–20–52	109–114	2.7	C	.2	.3	.....	.3	.6	.4	.7	.9	1.2	.3	.5	.8	1.1	1.5	1.4	1.7	1.5	1.9	2.0	2.2
Alfalfa, E23	115–120	3.1	C	.1	.3	.....	.2	.6	.3	.6	.8	1.1	.3	.5	.8	1.2	1.5	1.5	2.0	1.7	2.1	2.3	2.5
Alfalfa, E23 (in open)	295–300	C	.2	.2	.3	0	.3	.5	.5	.8	1.1	1.2	.4	.5	.8	1.3	1.6	1.6	2.1	1.7	2.0	2.4	2.6
Alfalfa, 162–98A	127–132	2.9	C	.2	.3	.....	.3	.8	.5	.9	1.3	1.7	.6	.8	1.1	1.7	2.2	2.1	2.7	2.4	2.6	2.8	2.5

SEPT. 5 TO 25

Item.	Pot No.	September.																				
		5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Evaporation (shallow tank), kgm. per sq. cm		12.08	7.58	8.62	7.37	7.65	10.15	9.67	13.12	5.63	9.85	14.98	7.74	7.89	12.14	10.76	13.82	10.15	6.06	9.36	8.50	12.48
Evaporation (deep tank), mm.		6.96	5.46	4.65	5.21	4.96	7.24	7.09	11.50	4.12	5.54	9.42	4.04	4.35	7.52	8.16	7.88	6.96	4.40	4.65	5.52	8.14
Evaporation (deep tank), inches		.274	.215	.183	.205	.195	.285	.279	.453	.162	.218	.371	.159	.171	.296	.321	.310	.274	.173	.183	.217	.320
Air temperature, max., °C		32.2	26.7	29.4	27.8	27.8	28.3	32.8	32.8	17.8	21.1	33.3	26.1	31.1	32.2	31.1	31.1	20.0	20.0	21.7	26.7	28.9
Air temperature, min., °C		10.0	11.1	8.9	13.9	10.0	9.4	13.3	5.0	—.6	1.7	6.7	6.1	12.8	15.6	12.2	12.8	—.6	2.8	3.9	8.3	8.3
Air temperature, integrated mean, °C		21.1	19.4	20.0	19.4	18.3	18.3	21.7	18.3	7.8	17.8	21.7	15.0	21.1	22.8	20.6	21.1	10.0	13.9	12.2	16.7	18.3
Air temperature, max., °F		90	80	85	82	82	83	91	91	64	70	92	79	88	90	88	88	68	68	71	80	84
Air temperature, min., °F		50	52	48	57	50	49	56	41	31	35	44	43	55	60	54	55	31	37	39	47	47
Air temperature, integrated mean, °F		70	67	68	67	65	65	71	65	46	64	71	59	70	73	69	70	50	57	54	62	65
Integrated radiation, cal. per sq. cm		714	573	642	573	564	813	753	707	492	823	753	731	630	808	776	738	692	722	842	820	750
Integrated wet-bulb depression, deg. hr.		226	117	81	56	74	178	173	213	86	122	262	146	140	208	169	196	124	126	123	194	223
Wind velocity, miles per hr		6.3	8.0	7.8	7.5	5.2	5.7	9.7	11.4	5.2	7.3	12.1	4.8	4.3	9.4	9.9	10.8	9.2	3.3	4.0	4.7	9.3
Wind velocity, meters per sec		2.8	3.6	3.5	3.4	2.3	2.5	4.3	5.1	2.3	3.3	5.4	2.1	1.9	4.2	4.4	4.8	4.1	1.5	1.8	2.1	4.2
Daily transpiration, kgm.:																						
Sudan grass	410–414	.9	.5	.6	.4	.5	.7	1.0	.9	.2	.2	1.1	.3	.6	.9	.9	1.0	.4	.2	.5	.5	.9
Sudan grass (in open)	319–323	1.0	.6	.7	.6	.6	.9	1.2	1.4	.2	.3	1.1	.4	.9	1.1	1.2	1.0	.6	.2	.2	.8	.9
Amaranthus	163–168	.4	.3	.5	.1	.2	.4	.5	.4	.2	.2	.6	.2	.4	.4	.4	.5	.2	.0	.2	.2	.5
Alfalfa, E23–20–52	109–114	2.5	2.1	2.0	1.7	1.9	2.5	2.9	3.3	1.5	1.8	3.2	2.1	2.7	3.7	3.4	3.6	2.6	2.0	2.4	2.7	3.5
Alfalfa, E23	115–120	2.9	2.3	2.4	2.0	2.4	3.0	3.3	3.9	1.8	2.3	3.9	2.6	3.2	4.6	4.3	4.4	3.1	2.4	2.7	3.2	4.1
Alfalfa, E23 (in open)	295–300	3.0	2.4	2.4	2.2	2.6	3.1	3.4	3.9	1.6	2.2	3.8	3.0	3.2	4.8	4.8	4.5	3.1	2.3	2.9	3.4	4.2
Alfalfa, 162–98A	127–132	3.7	3.0	2.6	2.7	3.1	3.4	4.2	4.9	2.3	2.6	4.5	3.7	4.0	5.7	5.3	5.4	3.7	3.1	3.5	3.2	4.6

55858°—16——2

The evaporation from the shallow tank as given in Table I is expressed in kilograms per square meter of water surface per day, calculated from the observed evaporation from a shallow blackened tank 6,540 sq. cm. in area and 2.5 cm. in depth, the depth of water being maintained automatically at about 1 cm. The evaporation from the deep tank (8 feet, or 243 cm., in diameter; depth of water, 50 cm.) is expressed in terms of the thickness of the layer of water evaporated each day, given both in inches and in millimeters. Since the loss of 1 kgm. of water from an area of 1 square meter represents a sheet of water 1 mm. in thickness, the daily evaporation from the two tanks is easily compared.

The maximum, minimum, and mean temperature of each day is given in Table I in both Fahrenheit and centigrade units. The mean daily temperature was determined by integrating the area bounded by the thermograph record with the aid of a planimeter, which gives a better representation of the mean temperature than the mean of the maximum and minimum values.

The daily radiation represents the total number of small calories received during the day on a surface 1 sq. cm. in area kept normal to the sun's rays. The radiation values given in Table I were computed from the records of a differential thermograph calibrated by means of a standardized Abbot silver-disk pyrheliometer.

The wet-bulb depression is expressed in hour degrees on both temperature scales and represents the summation of the depression for each hour of the day beginning at 5 a. m.

The mean wind velocity for the day as measured by a Robinson anemometer 3 feet above the ground is given in miles per hour and meters per second.

The daily values of the weather factors and the daily transpiration during the growing season of 1914 as given in Table I are plotted in figure 2. The graphs of the two evaporation tanks are seen to be similar though not identical. This similarity is of special interest when the difference in the hourly distribution of the evaporation from the two tanks is considered. The loss from the small shallow tank is confined almost wholly to the daylight hours, while the large deep tank shows a marked evaporation at night, due to the heat stored during the day in the large volume of water. The writers will show later that the daily transpiration is more closely correlated with the daily evaporation from the shallow tank than with that from the deep tank.

The daily transpiration is, of course, dependent not only upon the environment but upon the relative water requirement of the various species, the size of the plants, and the stage of growth. The daily transpiration is therefore supplemented in Table II with a statement of the period of growth, yield of dry matter, and water requirement of each variety. To this table has also been added the variety of plant used, the botanical name, and the pot numbers.

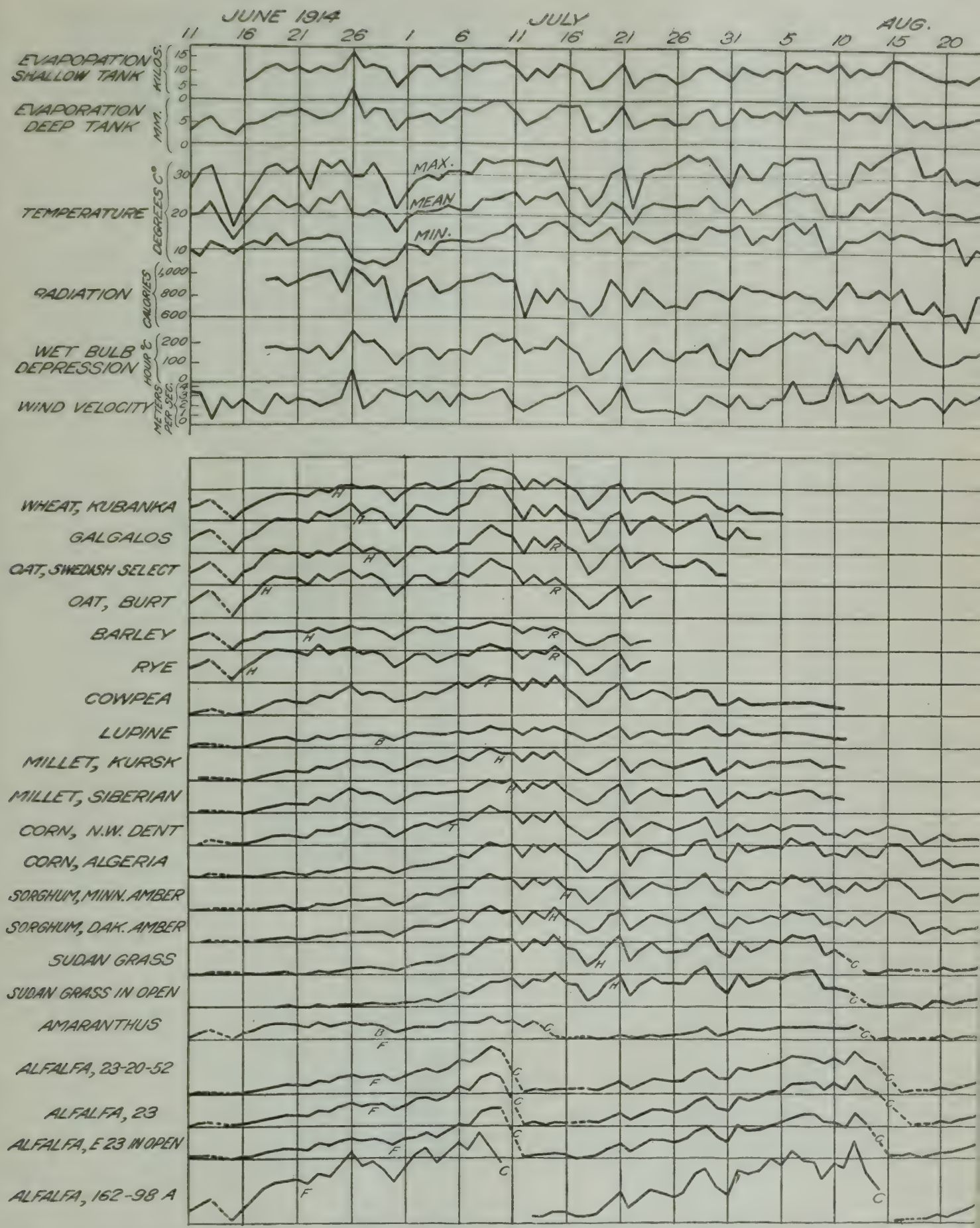

FIG. 2.—Graphs showing the daily intensity of environmental factors and the daily transpiration of 22 crops for the year 1914. The e per square meter. The evaporation from the large tank is given in millimeters. The temperatures are expressed in ° C.; the total radi on a surface normal to the sun's rays; the wet-bulb depression in hour degrees C.; the wind velocity in meters per second. The trans (zero) lines for each crop representing 3 kgm., except for alfalfas E23–20–52 and 162–98A, the base lines of which are 5 and 6 kgm., resp the graph indicates that the crop was cut and was followed by a new growth from the established root system. *H* signifies that ripening.

55858°—16. (To face page 164.)

TABLE II.—*Period of growth, yield, and water requirement of plants used in 1914 transpiration measurements*

Crop.	Variety.	Botanical name.	Period of growth.	Pot no.	Mean dry matter.	Mean water.	Mean grain.	Water requirement based on dry matter.
					Gm.	*Kgm.*	*Per cent.*	
Wheat	Kubanka (C. I. 1440)	*Triticum durum*	May 9–Aug. 6	1–6	306	160	38	518±6
Do	Galgalos (C. I. 2398)	*Triticum aestivum*	May 9–Aug. 11	7–12	298	186	33	624±5
Oat	Swedish Select (C. I. 134)	*Avena sativa*	May 9–Aug. 1	91–96	264	158	42	599±2
Do	Burt (C. I. 293)	do	May 9–July 25	97–102	251	154	42	615±6
Barley	Hannchen (C. I. 531)	*Hordeum distichon*	do	85–90	182	91	43	501±5
Rye	Spring (C. I. 73)	*Secale cereale*	do	103–108	195	121	27	622±7
Cowpea	S. P. I. 29282	*Vigna sinensis*	May 28–Aug. 12	169–174	170	112	34	659±5
Lupine	White (S. P. I. 35477)	*Lupinus albus*	do	175–180	90	76	18	870±34
Millet	Kursk (S. P. I. 34771)	*Chaetochloa italica*	June 9–Aug. 12	181–186	301	89	28	295±2
Do	Siberian (A. D. I. 3–4)	do	do	187–192	318	100	28	316±5
Corn	Northwestern Dent	*Zea mays*	June 3–Aug. 31	217–222	304	112	.....	368±6
Do	Algeria	do	do	238–242	417	137	.....	331±4
Sorghum	Minnesota Amber (A. D. I. 341–13)	*Andropogon sorghum*	June 9–Sept. 1	205–210	457	130	32	284±3
Do	Dakota Amber (A. D. I. 341–10–4)	do	do	211–216	427	126	34	296±1
Sudan grass:								
In screened inclosure—								
First crop	S. P. I. 25017	*Andropogon sorghum aethiopicus.*	June 10–Aug. 12	410–414	301	110	.....	366±3
Second crop			Aug. 13–Oct. 15	410–414	59	32	.....	540±6
Combined			June 10–Oct. 15	410–414	360	142	.....	394±4
In open—								
First crop			June 10–Aug. 12	319–323	216	88	.....	405±12
Second crop			Aug. 13–Oct. 14	319–323	61	38	.....	626±16
Combined			June 10–Oct. 14	319–323	277	126	.....	455±9
Amaranthus:								
First crop		*Amaranthus retroflexus*	June 3–July 14	163–168	124	39	.....	313±4
Second crop			July 15–Aug. 13	163–168	54	16	.....	304±3
Third crop			Aug. 14–Oct. 17	163–168	42	12	.....	287±4
Combined			June 3–Oct. 17	163–168	220	67	.....	306±1
Alfalfa:								
First crop	Grimm (A. D. I. E–23–20–52)	*Medicago sativa*	June 5–July 11	109–114	72	45	.....	619±5
Second crop			July 12–Aug. 15	109–114	66	60	.....	900±21
Third crop			Aug. 16–Oct. 26	109–114	120	130	.....	1,088±20
Combined			June 5–Oct. 26	109–114	258	234	.....	906±11

TABLE II.—*Period of growth, yield, and water requirement of plants used in 1914 transpiration measurements*—Continued

Crop.	Variety.	Botanical name.	Period of growth.	Pot no.	Mean dry matter.	Mean water.	Mean grain.	Water requirement based on dry matter.
					Gm.	*Kgm.*	*Per cent.*	
Alfalfa:								
In screened inclosure—								
First crop	Grimm (A. D. I. E-23)	*Medicago sativa*	June 5–July 11	115–120	87	53	.....	610±3
Second crop			July 12–Aug. 15	115–120	77	70	.....	906±14
Third crop			Aug. 16–Oct. 26	115–120	142	149	.....	1,055±12
Combined			June 5–Oct. 26	115–120	306	272	.....	890±6
In open—								
First crop			June 5–July 11	295–300	67	51	.....	767±13
Second crop			July 12–Aug. 14	295–300	58	67	.....	1,159±29
Third crop			Aug. 15–Oct. 27	295–300	142	159	.....	1,124±24
Combined			June 5–Oct. 27	295–300	267	276	.....	1,039±19
Alfalfa:								
First crop	Grimm (A. D. I. 162–98–A)	do	May 9–July 11	128–132	203	146	.....	720±5
Second crop			July 12–Aug. 15	128–132	146	118	.....	809±14
Third crop			Aug. 16–Oct. 26	128–132	209	187	.....	899±3
Combined			May 9–Oct. 26	128–132	557	452	.....	811±5

While large daily fluctuations occur in all the physical factors, the graphs do not show a marked "run" as the season advances. In other words, the seasonal change is not large. The evaporation shows a slight maximum in early August. The radiation is at its maximum in the latter part of June, at which time the sun reaches its greatest altitude. From this time onward the maximum intensity of the radiation (representing cloudless days) gradually decreases. The wind velocity, on the other hand, tends to increase slowly as the season advances. The maximum temperature and maximum wet-bulb depression occur during the middle of August.

MEASUREMENTS IN 1915

The daily transpiration measurements in 1915 were begun as soon as the crops were established and include, therefore, the whole growth period (Table III). The season was exceptionally rainy, which was unfortunate from the standpoint of the transpiration measurements, as the plants were often so wet in the morning that it was impossible to determine the transpiration of the preceding day. In such cases the mean transpiration has been given for the two or three days included in the rainy period. Such breaks in the daily record are indicated by dotted lines in figure 3, in which the data in Table III are presented graphically. Supplementary data relative to the plants used in the 1915 measurements are given in Table IV.

TABLE III.—*Measurements of environmental factors and daily transpiration, May 22 to September 20, 1915*

MAY 22 TO JUNE 20

Item.	Pot No.	May.						June.												
		22–23	24	25	26–27	28	29–31	1	2–4	5–6	7	8–9	10–11	12	13	14	15	16	17	18	19–20
Evaporation (deep tank) mm		3.40	2.95	5.62	5.92	2.79	2.57	0.56	3.56	8.43	3.71	4.09	6.53	8.66	3.10	4.57	4.04	6.20	5.46	5.82	3.91
Evaporation (deep tank) inches		.134	.116	.221	.233	.110	.101	.022	140	.332	.146	.161	.257	.341	.122	.180	.159	.124	.215	.229	.154
Air temperature, max. °C		22.2	25.0	24.4	14.4	18.3	16.7	21.7	18.3	13.9	20.0	23.9	23.3	21.7	15.6	21.1	25.6	23.3	25.6	27.8	22.2
Air temperature, min. °C		7.8	11.1	9.4	6.7	2.8	8.3	10.0	6.7	3.9	7.2	10.6	11.7	3.3	6.1	7.8	8.3	15.0	9.4	11.1	12.2
Air temperature, integrated mean. °C		15.0	19.4	16.7	10.0	11.1	11.7	16.1	12.8	8.3	13.3	16.7	17.8	12.2	10.0	14.4	17.2	18.3	17.8	18.3	16.1
Air temperature, max. °F		72	77	76	58	65	62	71	65	57	68	75	74	71	60	70	78	74	78	82	72
Air temperature, min. °F		46	52	49	44	37	47	50	44	39	45	51	53	38	43	46	47	59	49	62	54
Air temperature, integrated mean. °F		59	67	62	50	52	53	61	55	47	56	62	64	54	50	58	63	65	64	65	61
Wind velocity miles per hr		5.2	5.0	10.1	16.6	7.4	4.8	7.3	4.4	7.5	7.4	7.0	10.6	11.0	4.3	5.3	4.9	10.8	7.0	8.6	5.7
Wind velocity meters per sec.		2.3	2.2	4.5	7.4	3.3	2.1	3.3	2.0	3.4	3.3	3.1	4.7	4.9	1.9	2.4	2.2	4.8	3.1	3.8	2.5
Daily transpiration, kgm.:																					
Wheat, Kubanka	1–6	.1	.2	.1	.....	.1	.1	.1	.1	.....	.5	.4	.6	.7	.4	.7	1.0	1.1	1.1	1.0	.8
Wheat, Galgalos	7–12	.1	.2	.1	.....	.1	.1	.2	.2	.....	.5	.5	.7	.7	.2	.9	1.0	1.0	1.1	1.1	.8
Wheat, Washington Bluestem	13–18	.0	.1	.1	.....	.1	.0	.1	.1	.....	.3	.3	.5	.6	.3	.6	1.0	1.0	.9	1.2	.9
Wheat, Turkestan	19–24	.0	.0	.1	.....	.0	.0	.1	.1	.....	.3	.4	.7	.7	.3	.8	1.3	1.2	1.1	1.6	1.0
Wheat, Marquis	103–108	.0	.0	.2	.....	.1	.0	.1	.2	.....	.4	.5	.7	.6	.2	.9	1.1	1.1	.9	1.2	.8
Wheat, Kubanka	109–114	.....	.1	.2	.....	.1	.0	.0	.2	.....	.3	.4	.7	.6	.3	.8	1.0	1.1	.9	1.2	.9
Wheat, Preston	127–132	.....	.....	.....	.....	.0	.....	.2	.1	.....	.2	.4	.6	.4	.3	1.2	1.0	1.1	1.3	1.1	1.1
Oat, Swedish Select	139–144	.....	.....	.....	.....	.0	.0	.1	.1	.....	.2	.3	.5	.3	.2	1.0	.8	1.0	1.1	1.2	1.0
Oat, Burt	145–150	.....	.....	.....	.....	.0	.0	.1	.....	.....	.1	.2	.3	.2	.5	.6	.8	.8	1.0	.9	.9
Barley, Hannchen	133–138	.....	.....	.....	.....	.0	.0	.1	.1	.....	.2	.3	.5	.2	.2	1.2	.9	.9	1.1	1.1	1.0
Rye, spring	151–156	.....	.....	.....	.....	.0	.0	.1	.....	.....	.2	.3	.5	.4	.7	.8	1.1	1.0	1.2	1.1	1.1
Flax, North Dakota (C. I. 13)	157–162	.....	.....	.....	.....	.....	.....	.0	.....	.....	.0	.0	.2	.....	.4	.1	.4	.3	.5	.2	.5
Flax, North Dakota (C. I. 19)	187–192	.....	.....	.....	.....	.....	.....	.0	.....	.....	.0	.0	.2	.....	.3	.3	.4	.3	.5	.3	.5
Flax, Smyrna	163–168	.....	.....	.....	.....	.....	.....	.0	.....	.....	.0	.0	.2	.....	.4	.2	.4	.3	.5	.3	.4
Cowpea	217–222	.....	.....	.....	.....	.....	.....	.....	.....	.....	.0	.....	.1	.1	.1	.....	.1	.....	.0	.2	.....
Alfalfa, E–23	193–198	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.0	.2	.0
Alfalfa, 162–98A1	199–204	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.2	.....

JUNE 21 TO JULY 11

Item.	Pot No.	June.									July.										
		21–22	23	24	25	26	27	28	29	30	1	2	3	4	5	6	7	8	9	10	11
Evaporation (shallow tank), kgm. per sq. meter											7.10	2.77	5.80	6.26	6.98	6.28	6.39	4.82	9.19	8.08	8.21
Evaporation (deep tank) mm		5.87	5.64	0.86	5.77	5.03	6.56	3.25	6.20	5.74	8.13	3.46	5.46	3.76	3.15	8.86	4.60	4.22	6.43	6.48	5.82
Evaporation (deep tank) inches		.231	.222	.034	.227	.198	.258	.128	.244	.226	.320	.136	.215	.148	.124	.349	.181	.166	.253	.255	.229
Air temperature, max. °C		21.7	21.7	23.3	27.2	27.8	27.2	21.7	27.2	22.2	26.7	16.7	23.9	21.1	29.4	32.8	25.0	28.9	30.6	26.7	35.0
Air temperature, min. °C		11.7	11.7	11.7	8.3	10.6	11.1	12.8	11.7	11.1	8.9	9.4	4.4	7.2	13.3	10.0	14.4	11.7	12.2	16.1	14.4
Air temperature, integrated mean. °C		16.7	17.2	17.2	18.3	18.9	18.3	16.7	17.8	16.7	17.2	13.3	12.2	13.9	20.6	18.9	19.4	18.3	21.7	21.1	25.0
Air temperature, max. °F		71	71	74	81	82	81	71	81	72	80	62	75	70	85	91	77	84	87	80	95
Air temperature, min. °F		53	53	53	47	51	52	55	53	52	48	49	40	45	56	50	58	53	54	61	58
Air temperature, integrated mean. °F		62	63	63	65	66	65	62	64	62	63	56	54	57	69	66	67	65	71	70	77
Integrated radiation, cal. per sq. cm										1,015	805	667	764	1,132	963	751	1,013	616	984	904	981
Integrated wet-bulb depression, hr. deg. C									27	45			84	104	111	132	94	66	115	127	142
Wind velocity miles per hr		8.7	5.1	5.4	8.0	3.8	7.0	5.7	3.5	5.4	8.3	3.5	8.0	2.9	3.7	6.9	5.7	5.2	8.0	6.8	5.5
Wind velocity meters per second		3.9	2.3	2.4	3.6	1.7	3.1	2.5	1.6	2.4	3.7	1.6	3.6	1.3	1.7	3.1	2.5	2.3	3.6	3.0	2.5
Daily transpirations, kgm.:																					
Wheat, Kubanka	1–6	.9	.8	1.2	1.4	1.5	1.5	1.0	1.2	1.8	1.7	.9	1.0	1.2	1.7	1.9	1.7	1.8	1.9	2.1	2.4
Wheat, Galgalos	7–12	.9	.8	1.1	1.4	1.6	1.6	.8	1.3	1.7	1.8	.8	1.1	1.1	1.4	1.8	1.6	1.6	2.2	2.2	2.7
Wheat, Washington Bluestem	13–18	.9	.8	1.1	1.5	1.5	1.7	.8	1.4	1.7	1.6	.8	1.2	1.1	1.5	1.9	1.6	1.6	2.3	2.5	2.8
Wheat, Turkestan	19–24	1.1	1.0	1.3	1.9	2.1	2.3	1.3	1.8	2.5	2.6	1.1	1.8	1.8	2.4	2.4	2.3	2.2	3.1	3.0	3.1
Wheat, Marquis	103–108	.9	.8	1.2	1.4	1.5	1.6	.9	1.2	1.7	1.8	.8	1.0	1.2	1.6	1.9	1.7	1.6	2.1	2.1	3.0
Wheat, Kubanka	109–114	.9	1.0	1.1	1.4	1.7	1.8	1.0	1.5	1.9	2.0	.9	1.3	1.6	1.9	2.1	2.0	1.8	2.4	2.5	2.9
Wheat, Preston	127–132	1.1	1.1	1.3	1.5	1.9	2.0	1.1	1.7	2.3	2.3	1.0	1.4	1.7	2.4	2.4	2.0	2.0	2.8	3.0	3.7
Oat, Swedish, Select	139–144	1.2	1.1	1.4	2.2	2.2	2.4	1.3	2.0	2.7	2.8	1.1	1.7	1.9	2.6	2.7	2.4	2.3	3.8	3.5	4.6
Oat, Burt	145–150	1.1	1.1	1.3	1.6	2.2	2.1	1.1	1.6	2.2	2.3	.8	1.5	1.5	2.3	2.4	2.1	2.0	2.9	2.9	3.7
Barley, Hannchen	133–138	1.2	1.2	1.5	1.6	2.5	2.0	1.3	1.7	2.3	2.2	1.1	1.5	1.6	2.3	2.2	2.0	2.1	3.0	3.2	3.8
Rye, spring	151–156	1.0	1.0	1.4	1.6	2.4	2.1	1.2	1.5	2.1	2.2	.9	1.3	1.5	1.9	2.2	1.7	1.6	2.3	2.3	3.1
Flax, North Dakota (C. I. 13)	157–162	.5	.6	.7	.8	1.5	1.3	.8	1.1	1.6	1.4	.6	1.0	1.0	1.9	1.9	1.9	1.7	1.5	2.4	3.6
Flax, North Dakota (C. I. 19)	187–192	.6	.5	.8	1.0	1.7	1.4	.9	1.2	2.0	2.1	.8	1.3	1.5	2.3	2.7	2.3	2.3	3.5	3.2	4.4
Flax, Smyrna	163–168	.5	.6	.6	.9	1.5	1.5	.7	1.1	1.7	1.7	.8	1.2	1.4	2.0	2.5	2.2	1.9	3.3	3.2	4.1
Cowpea	217–222	.0	.2	.2	.....	.4	.3	.....	.1	.5	.1	.....	.2	.2	.3	.4	.3	.7	.3	.8	.9
Millet	223–228	.....	.....	.....	.....	.....	.2	.....	.....	.2	.....	.....	.2	.1	.2	.3	.3	.4	.3	.6	.8
Sorghum	235–240	.....	.....	.....	.....	.....	.2	.....	.....	.2	.1	.....	.2	.0	.2	.2	.1	.3	.0	.2	.2
Corn	241–246	.....	.....	.....	.....	.....	.2	.....	.....	.5	.....	.3	.0	.0	.1	.2	.1	.2	.1	.3	.4
Potato	307–312	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.4	.0	.....	.3	.1	.2	.3	.2	.3	.5
Amaranthus	211–216	.....	.....	.....	.....	.....	.4	.....	.1	.2	.3	.....	.3	.2	.4	.7	.6	.6	.6	.9	1.5
Sudan grass	253–258	.....	.....	.....	.....	.....	.1	.....	.....	.3	.....	.3	.1	.....	.2	.1	.2	.1	.1	.3	.5
Alfalfa, E–23	193–198	.1	.3	.4	.1	.8	.4	.2	.3	.8	.7	.4	.5	.7	1.0	1.4	1.2	1.3	1.6	1.9	2.5
Alfalfa, 162–98A1	199–204	.1	.2	.3	.2	.5	.3	.1	.2	.5	.6	.2	.4	.5	.8	1.1	1.0	1.0	1.4	1.7	2.1

TABLE III.—*Measurements of environmental factors and daily transpiration, May 22 to September 20, 1915*—Continued

JULY 12 TO AUG. 4

Item.	Pot No.	July.																August.			
		12	13	14	15	16	17–19	20	21	22	23	24	25	26–27	28	29	30	31	1	2	3	4
Evaporation (shallow tank) kgm. per sq. meter	.....	8.51	7.18	8.93	.....	5.95	5.72	2.49	6.54	8.87	7.98	5.90	6.00	3.95	5.88	7.05	5.40	6.80	4.68	5.69	7.70	6.54
Evaporation (deep tank) mm	.....	6.35	7.77	7.22	4.52	8.74	4.98	2.16	4.85	7.09	6.65	4.68	5.69	3.00	4.57	6.35	5.41	5.84	4.39	4.52	5.74	6.07
Evaporation (deep tank) inches	.....	.250	.306	.284	.178	.344	.196	.085	.191	.279	.262	.184	.224	.118	.180	.250	.213	.230	.173	.178	.226	.239
Air temperature, max. °C	.....	28.9	31.1	32.2	27.8	28.9	25.6	17.2	31.7	30.6	33.3	27.8	25.0	23.9	28.3	29.4	26.1	27.2	26.7	21.7	26.7	28.3
Air temperature, min. °C	.....	13.3	11.7	16.1	13.3	13.9	12.2	11.1	14.4	12.2	13.9	8.9	14.4	15.0	13.3	10.6	13.3	11.7	10.6	9.4	13.3	11.1
Air temperature, integrated mean °C	.....	21.7	21.1	23.9	20.6	20.0	17.2	14.4	23.3	21.1	23.9	19.4	18.3	18.3	20.6	18.3	20.0	18.9	15.6	15.6	17.8	19.4
Air temperature, max. °F	.....	84	88	90	82	84	78	63	89	87	92	82	77	75	83	85	79	81	80	71	80	83
Air temperature, min. °F	.....	56	53	61	56	57	54	52	58	54	57	48	58	59	56	51	56	53	51	49	56	52
Air temperature, integrated mean °F	.....	71	70	75	69	68	63	58	74	70	75	67	65	65	69	65	68	66	60	60	64	67
Integrated radiation, cal. per sq. cm	.....	984	806	1,013	764	645	583	417	1,081	852	793	625	906	570	706	729	694	769	622	860	919	724
Integrated wet-bulb depression, hr. deg. C	.....	106	101	144	105	89	93	52	103	160	162	79	64	39	63	77	61	84	53	77	77	110
Wind velocity, miles per hr	.....	5.0	6.7	6.0	7.5	8.5	6.7	5.6	5.0	6.7	5.6	5.2	8.9	5.4	4.8	4.9	7.3	7.6	5.4	8.0	2.6	4.4
Wind velocity, meters per sec.	.....	2.2	3.0	2.7	3.4	3.8	3.0	2.5	2.2	3.0	2.5	2.3	4.0	2.4	2.1	2.2	3.3	3.4	2.4	3.6	1.2	2.0
Daily transpiration, kgm.:																						
Wheat, Kubanka	1–6	2.3	2.0	2.6	2.1	1.6	1.5	.7	2.1	2.3	2.4	1.3	1.6	1.0	1.2	1.8	1.2	1.7	.6	1.0	1.1	1.4
Wheat, Galgalos	7–12	2.5	2.2	2.8	2.2	1.7	1.5	.5	1.9	2.4	2.5	1.1	1.2	.9	1.1	1.6	.9	1.4	.6	.8	.9	1.2
Wheat, Washington Blue-stem	13–18	2.8	2.7	3.2	2.6	2.0	1.8	.8	2.3	2.9	3.2	1.7	1.8	1.2	1.6	2.2	1.5	2.1	1.3	1.4	1.6	1.8
Wheat, Turkestan	19–24	3.0	2.9	4.0	3.0	2.4	2.2	.8	2.5	3.4	3.3	1.6	1.8	1.2	1.9	2.0	1.5	2.0	.7	1.0	1.4	1.6
Wheat, Marquis	103–108	2.8	2.3	3.0	2.3	1.7	1.7	.5	2.0	2.6	2.7	1.4	1.6	1.0	1.5	1.9	1.2	1.8	.7	.9	1.3	1.5
Wheat, Kubanka	109–114	2.8	2.4	3.3	2.5	2.0	1.9	.8	2.5	3.0	3.2	1.7	1.9	1.3	1.7	2.2	1.6	2.2	1.1	1.3	1.8	2.2
Wheat, Preston	127–132	3.1	2.7	3.8	3.3	2.2	2.2	.8	2.8	3.4	3.4	2.0	2.2	1.6	2.1	2.4	1.9	2.5	1.3	1.4	2.0	2.4
Oat, Swedish select	139–144	4.5	4.0	5.4	4.4	3.4	3.2	.9	3.1	4.0	3.8	2.0	2.3	1.5	2.3	3.0	2.2	3.3	1.2	2.0	3.1	3.2
Oat, Burt	145–150	3.7	3.1	4.2	3.2	2.5	2.3	.7	2.6	3.5	3.4	1.8	2.0	1.2	1.9	2.2	1.8	2.0	.7	.9	1.5	1.3
Barley, Hannchen	133–138	3.6	3.1	4.3	3.6	2.8	2.4	.9	2.6	3.0	3.1	1.9	1.9	1.2	1.6	2.0	1.6	2.1	.5	.8	1.2	1.5
Rye, spring	151–156	2.3	2.4	3.0	2.4	1.7	1.7	.4	2.0	2.6	2.7	1.3	1.5	1.0	1.5	1.6	1.2	1.6	.8	1.0	1.3	1.5
Flax, North Dakota (C. I. 13)	157–162	3.1	2.6	3.5	2.9	2.0	2.1	.6	2.5	2.9	2.9	1.6	1.7	1.2	1.7	2.0	1.5	2.1	.7	1.2	1.8	1.5
Flax, North Dakota (C. I. 19)	187–192	4.2	3.5	4.7	3.9	2.9	2.8	.9	3.2	3.8	2.8	1.5	2.1	1.2	2.2	2.4	2.2	2.7	1.2	1.6	2.3	2.0
Flax, Smyrna	163–168	3.8	3.3	4.7	3.9	3.0	2.9	1.1	3.4	4.0	3.2	1.8	2.3	1.2	1.5	2.3	2.0	2.4	1.2	1.9	2.4	2.0
Cowpea	217–222	1.1	1.0	1.3	1.3	.9	1.0	.3	1.3	1.5	2.0	.9	1.0	.7	1.2	1.6	.9	1.6	1.0	.8	1.1	1.1
Millet	223–228	.9	.9	1.3	1.1	.8	1.0	.3	1.4	1.5	2.2	.9	1.1	.7	1.2	1.6	.9	1.5	.5	.7	1.4	1.3
Sorghum	235–240	.4	.4	.5	.4	.2	.4	.1	1.0	1.1	1.0	.4	.6	.4	.8	1.1	.5	1.1	.4	.5	1.1	1.0
Corn	241–246	.5	.3	1.0	.5	.3	.6	.1	.5	1.0	1.1	.2	.6	.2	.5	.9	.4	.7	.2	.3	.7	.7
Potato	307–312	.3	.3	.6	.3	.1	.3	.....	.2	1.1	.7	.4	.7	.3	.6	.8	.2	.6	.3	.1	.5	.4
Amaranthus	211–216	1.0	1.4	1.4	1.2	.8	.8	.2	1.2	1.3	1.5	.8	.8	.6	.9	1.5	.6	1.1	.3	.8	.8	1.0
Sudan grass	253–258	.5	.5	1.1	.8	.7	.9	.1	1.0	1.4	1.9	1.0	1.0	.7	1.0	1.8	.8	1.6	.8	.7	1.3	1.2
Alfalfa, E-23	193–198	2.5	2.5	3.1	2.9	2.0	2.2	1.0	3.2	3.7	4.0	2.4	2.7	1.7	2.4	2.9	2.8	3.6	1.7	1.9	2.3	2.3
Alfalfa, 162–98A1	199–204	2.0	2.2	2.7	2.5	1.7	1.9	.8	2.6	3.0	2.8	1.9	2.3	1.5	2.3	2.6	2.4	3.1	1.5	2.1	2.5	2.4

Item.	Pot No.	August.																				
		5	6–8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Evaporation (shallow tank), kgm. per sq. meter	.....	8.41	3.40	6.28	6.46	6.36	7.02	5.58	5.43	4.91	5.88	4.80	5.32	4.88	3.46	4.10	4.13	3.61	4.50	5.82	3.47	6.04
Evaporation (deep tank) mm.	.....	7.08	4.72	4.78	4.60	5.66	.33	4.34	4.90	3.68	4.39	5.15	4.62	3.96	4.06	4.42	3.61	5.39	3.81	5.26	1.98	4.78
Evaporation (deep tank) inches.	.....	.279	.186	.188	.181	.223	210	.171	.193	.145	.173	.203	.182	.156	.160	.174	.142	.212	.150	.207	.078	.188
Air temperature, max. °C.	.....	31.7	24.4	25.6	27.8	27.8	28.3	26.7	28.3	24.4	27.2	26.7	23.9	24.4	25.0	26.1	27.8	23.3	23.9	26.1	22.8	26.7
Air temperature, min. °C.	.....	13.9	12.2	11.7	11.7	12.8	12.2	12.8	12.2	13.3	12.8	11.1	13.3	10.6	11.1	10.6	11.7	9.4	12.2	11.1	11.1	13.3
Air temperature integrated, mean °C.	.....	23.3	17.8	17.8	19.4	19.4	19.4	19.4	17.8	17.8	18.9	16.7	17.2	15.0	16.7	18.3	18.3	15.6	16.7	17.2	15.6	19.4
Air temperature, max. °F.	.....	89	76	78	82	82	83	80	83	76	81	80	75	76	77	79	82	74	75	79	73	80
Air temperature, min. °F.	.....	57	54	53	53	55	54	55	54	56	55	52	56	51	52	51	53	49	54	52	52	56
Air temperature integrated, mean °F.	.....	74	64	64	67	67	67	67	64	64	66	62	63	59	62	65	65	60	62	63	60	67
Integrated radiation, cal. per sq. cm	.....	953	477	892	825	727	793	815	690	672	741	628	648	469	618	754	648	497	593	570	487	773
Integrated wet-bulb depression, hr. deg. C.	.....	169	52	92	98	96	119	94	81	60	72	42	41	41	45	74	90	61	41	82	39	105
Wind velocity miles per hr.	.....	5.0	3.8	3.9	3.1	4.4	5.5	2.3	4.1	3.4	3.4	4.0	4.5	3.1	6.6	1.6	4.4	3.3	6.3	5.0	2.9	3.5
Wind velocity meters per sec.	.....	2.2	1.7	1.7	1.4	2.0	2.5	1.0	1.8	1.5	1.5	1.8	2.0	1.4	2.9	.7	2.0	1.5	2.8	2.2	1.3	1.6
Daily transpiration, kgms.:																						
Wheat, Kubanka	1–6	1.6	.2	1.6	.1	.2	.8	.3	.....	.2	.2	.0	.1	.3	.3	.....	.....	.....	.....	.....	.....	.....
Wheat, Galgalos	7–12	1.4	.1	1.3	.3	.....	.6	.3	.....	.1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Wheat, Washington Blue-stem	13–18	2.4	.8	2.3	1.4	.8	1.8	1.3	1.0	.7	.9	.2	.4	.3	.3	.4	.2	.....	.4	.1	.....	.....
Wheat, Turkestan	19–24	1.8	.1	1.5	.9	.3	1.1	.7	.....	.4	.4	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Wheat, Marquis	103–108	1.4	.2	1.3	.8	.1	.7	.5	.....	.3	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Wheat, Kubanka	109–114	2.5	.5	1.9	1.1	.5	1.4	1.0	.3	.5	.6	.1	.3	.3	.3	.3	.2	.....	.....	.....	.....	.....
Wheat, Preston	127–132	2.8	.7	2.1	1.5	1.0	1.9	1.5	.6	1.0	.9	.4	.5	.5	.3	.7	.3	.....	.3	.....	.....	.....
Oat, Swedish Select	139–144	4.0	.7	3.1	1.4	.8	1.5	1.2	.3	.8	.8	.1	.3	.4	.3	.....	.....	.....	.....	.....	.....	.....
Oat, Burt	145–150	1.7	.2	1.3	.6	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Barley, Hannchen	133–138	1.3	.....	1.0	.3	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Rye, Spring	151–156	1.6	.4	1.5	1.0	.3	.8	1.0	.1	.9	.5	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Flax, North Dakota (C. I. 13)	157–162	1.9	.1	1.4	.8	.1	.6	.7	.....	.6	.4	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Flax, North Dakota (C. I. 19)	187–192	2.4	.6	2.2	1.0	.3	1.4	1.1	.3	.8	.6	.0	.2	.3	.2	.4	.1	.....	.2	.2	.....	.....
Flax, Smyrna	163–168	2.9	.8	2.4	1.2	.5	1.8	1.6	.2	.8	.4	.1	.3	.4	.1	.1	.3	.....	.2	.0	.....	.....
Cowpea	217–222	1.5	.4	1.7	.9	.8	1.3	1.4	1.0	1.0	1.1	1.0	1.1	.6	.7	.8	.4	.1	.5	.5	.2	1.0
Millet	223–228	1.7	.....	1.8	1.1	1.1	1.7	1.1	.....	1.1	1.1	.5	.9	.6	.5	1.1	.7	.0	.7	.7	.4	1.3
Sorghum	235–240	1.5	.6	1.5	.9	.5	1.0	1.0	.5	1.0	.7	.5	.7	.5	.3	.6	.5	.....	.6	.3	.2	1.1
Corn	241–246	1.0	.1	1.2	.5	.3	.8	.4	.2	.7	.5	.2	.4	.3	.2	.5	.4	.1	.5	.3	.1	.8
Potato	307–312	.3	.....	1.2	.3	.2	.2	.6	.....	.5	.5	.2	.1	.3	.0	.5	.4	.1	.4	.1	.0	.7
Amaranthus	211–216	1.4	C.	.6	.....	.....	.0	.1	.....	.3	.2	.0	.1	.1	.0	.2	.2	.1	.3	.2	.1	.5
Sudan grass	253–258	1.9	.6	1.8	.9	.6	1.3	1.0	.8	1.0	.5	.7	.6	.3	.2	.6	.5	.1	.4	.5	.1	C.
Alfalfa, E–23	193–198	3.6	C.	.8	.0	.1	.3	.5	.2	.7	.8	.6	.8	.5	.9	1.3	1.3	.7	1.1	1.4	.8	2.3
Alfalfa, 162–98A1	199–204	3.4	C.	.6	.....	.1	.3	.5	.1	.7	.9	.6	.7	.7	1.0	1.4	1.3	.9	1.2	1.5	.8	2.1

TABLE III.—*Measurements of environmental factors and daily transpiration, May 22 to September 20, 1915*—Continued

AUG. 28 TO SEPT. 20

Item.	Pot No.	August.				September.															
		28	29	30	31	1	2	3	4	5	6	7	8	9	10–11	12–13	14–15	16	17	18	19	20
Evaporation (shallow tank), kgm. per sq. meter		5.81												9.37	6.06	5.93	4.71	6.94	6.51	8.63	7.28	5.32
Evaporation (deep tank) mm		6.22	4.09	5.84	4.93	8.18	7.77	7.46	5.44	4.52	4.85	7.75	2.01	6.93	4.39	4.85	4.47	3.30	3.66	6.02	5.18	4.98
Evaporation (deep tank) inches		.245	.161	.230	.194	.322	.306	.294	.214	.178	.191	.305	.079	.273	.173	.191	.176	.130	.144	.237	.204	.196
Air temperature, max., °C		24.4	22.2	26.1	34.4	33.3	28.9	28.9	27.8	23.9	26.7	31.1	20.6	25.6	25.0	25.0	15.6	25.0	28.3	27.2	30.0	17.2
Air temperature, min., °C		3.9	5.6	9.4	13.3	7.2	10.6	11.7	12.8	8.9	11.7	11.1	10.6	8.3	8.3	6.1	7.8	10.0	7.8	13.3	5.0	2.8
Air temperature, integrated mean, °C		14.4	13.3	17.8	22.8	19.4	20.6	20.0	19.4	14.4	17.8	18.9	13.3	16.7	16.1	13.9	10.6	15.6	17.2	18.9	17.8	11.1
Air temperature, max., °F		76	72	79	94	92	84	84	82	75	80	88	69	78	77	77	60	77	83	81	86	63
Air temperature, min., °F		39	42	49	56	45	51	53	55	48	53	52	51	47	47	43	46	50	46	56	41	37
Air temperature, integrated mean, °F		58	56	64	73	67	69	68	67	58	64	66	56	62	61	57	51	60	63	66	64	52
Integrated radiation, cal. per sq. cm		548	825	805	776	822	808	758	645	591	729	662	432	706	515	600	618	790	731	734	603	813
Integrated wet-bulb depression, hr. deg. C		140	123	111	146	223	167	124	121	62	63	.....	48	84	107	53	89	113	163	134	183	102
Wind velocity miles per hour		5.4	6.9	5.8	3.9	5.8	8.7	10.0	6.8	5.3	5.7	7.2	5.5	10.9	6.8	7.0	5.7	3.9	2.5	7.0	4.1	4.5
Wind velocity meters per sec.		2.4	3.1	2.6	1.7	2.6	3.9	4.5	3.0	2.4	2.5	3.2	2.5	4.9	3.0	3.1	2.5	1.7	1.1	3.1	1.8	2.0
Daily transpiration, kgm.:																						
Cowpea	217–222	.5	.3	.....	.3	.6	.4	.2	.1	.....	.1	.3	0	.....	.....	.....	.....	.....	.....	.....	.....	.....
Millet	223–228	.8	.6	.5	1.0	1.2	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Sorghum	235–240	.5	.5	.4	.9	1.4	1.1	.8	.7	.2	.6	1.3	.1	.8	.4	.5	.....	.....	.....	.....	.....	.....
Corn	241–246	.5	.1	.1	.5	.6	.4	.2	.3	.....	.2	.5	.1	.2	.2	.4	.....	.....	.....	.....	.....	.....
Potato	307–312	.2	.1	.1	.3	.3	.4	.2	.2	.1	.1	.4	.1	.1	.1	.4	.....	.....	0	.7	.2	.1
Amaranthus	211–216	.4	.1	.....	.2	.4	.3	.4	.1	.1	.2	.4	.1	.2	.2	.2	0	.2	.3	.5	.3	.1
Sudan grass	253–258	.2	.3	.....	.1	.3	.2	.1	.3	.1	.1	.5	.1	.3	.2	.3	.1	.1	.2	.9	.3	.1
Alfalfa, E–23	193–198	2.3	1.4	1.3	2.7	3.7	3.0	2.1	2.6	1.4	2.6	3.4	1.0	1.8	1.8	1.2	.5	1.6	2.2	2.7	2.7	1.7
Alfalfa, 162–98A1	199–204	2.4	1.2	1.3	2.6	3.4	3.0	2.0	2.3	1.3	2.3	3.0	1.0	2.1	1.8	1.3	.7	1.5	2.0	2.6	2.5	1.9

TABLE IV.—*Period of growth, yield, and water requirement of plants used in 1915 transpiration measurements*

Crop.	Variety.	Botanical name.	Period of growth.	Pot No.	Mean dry matter.	Mean water.	Mean grain.	Water requirements based on dry matter.
					Gm.	*Kgm.*	*Per cent.*	
Wheat	Kubanka (C. I. 1440)	*Triticum durum*	May 22–Aug. 21	1–6	209	85	32	405±6
Do	Galgalos (C. I. 2398)	*Triticum aestivum*	May 22–Aug. 16	7–12	168	81	32	481±4
Do	Washington Bluestem (C. I. 4067).	do	May 22–Aug. 26	13–18	215	105	15	491±4
Do	Turkestan (C. I. 4087)	do	May 22–Aug. 18	19–24	219	111	30	505±3
Do	Marquis (C. I. 3641)	do	May 22–Aug. 16	103–108	208	88	33	424±3
Do	Kubanka (C. I. 1440)	*Triticum durum*	May 24–Aug. 24	109–114	267	108	34	406±3
Do	Preston (C. I. 2958)	*Triticum aestivum*	May 28–Aug. 26	127–132	279	126	26	452±6
Oat	Swedish Select (C. I. 134)	*Avena sativa*	May 28–Aug. 21	139–144	332	148	41	448±10
Do	Burt (C. I. 293)	do	May 28–Aug. 11	145–150	233	103	38	445±5
Barley	Hannchen (C. I. 531)	*Hordeum distichon*	do	133–138	262	106	43	404±11
Rye	Spring (C. I. 73)	*Secale cereale*	May 28–Aug. 17	151–156	203	95	32	469±8
Flax	North Dakota (C. I. 13)	*Linum usitatissimum*	June 1–Aug. 17	157–162	149	86	29	579±10
Do	North Dakota (C. I., 19)	do	June 1–Aug 26	187–192	186	114	26	615± 7
Do	Smyrna (C. I., 30)	do	do	163–168	182	121	24	663±16
Cowpea	S. P. I., 29282	*Vigna sinensis*	June 4–Sept. 9	217–222	128	53	33	413± 5
Millet	Kursk (S. P. I., 34771)	*Chaetochloa italica*	June 26–Sept. 2	223–228	256	52	31	202± 1
Sorghum	Minnesota Amber (A. D. I., 341–13.)	*Andropogon sorghum*	June 26–Sept. 14	235–240	203	41	19	203± 3
Corn	Northwestern Dent	*Zea mays*	do	241–246	112	28	.....	253± 7
Potato	Irish Cobbler	*Solanum tuberosum*	July 1–Sept. 25	307–312	67	22	.....	329± 4
Amaranthus:								
First crop		*Amaranthus retroflexus*	June 26–Aug. 6	211–216	129	31	.....	239± 3
Second crop			Aug. 6–Sept. 21	211–216	30	6	.....	188± 4
Combined			June 26–Sept. 21	211–216	159	36	.....	229± 3
Sudan grass:								
First crop	S. P. I., 25071	*Andropogon sorghum aethiopicus.*	June 26–Aug. 28	253–258	176	43	.....	246± 3
Second crop			Aug. 29–Sept. 24	253–258	15	6	.....	424±12
Combined			June 26–Sept. 24	253–258	190	49	.....	260± 3
Alfalfa:								
First crop	Grimm (A. D. I., E-23)	*Medicago sativa*	June 17–Aug. 6	193–198	133	86	.....	646±14
Second crop			Aug. 7–Sept. 21	193–198	83	65	.....	773± 6
Combined			June 17–Sept. 21	193–198	217	151	.....	696±10
Alfalfa:								
First crop	Grimm (A.D.I., 162–98–A–1)	do	June 17–Aug. 6	199–204	115	70	.....	619±13
Second crop			Aug. 7–Sept. 21	199–204	78	60	.....	782±16
Combined			June 17–Sept. 21	199–204	192	131	.....	685±13

COMPARISON OF THE TRANSPIRATION OF THE DIFFERENT CROPS

The graphs in figures 2 and 3 illustrate in a striking manner the great fluctuations in the water required daily by plants to maintain normal growth. During the period from July 8 to 10, 1914, for example, the wheats under observation required about three times as much water each day as during the period from July 17 to 19. In 1914 the maximum rate of transpiration of the series as a whole occurred about July 9, at which period the grains were headed, the legumes were in bloom or coming into bloom, and corn was beginning to tassel.

Alfalfa, Sudan grass, and amaranthus, of which several cuttings were made from the same root system, showed a continuous increase in the transpiration rate up to the time of cutting. The transpiration of Minnesota Amber sorghum, Algeria corn, and lupine was relatively uniform from about July 9 to near the end of the growth season. Among the small grains barley and rye showed the least change in the transpiration rate. The grain crops were harvested at the stage when similar crops in the field are cut with the binder, and it is interesting to note at this time transpiration was approximately one-fourth to one-half the maximum rate.

The season of 1915, like that of 1914, shows a nearly uniform evaporation rate throughout the more active growth period if the daily fluctuations are ignored. The season was very rainy. This is reflected in the radiation graph which is far more irregular than in 1914, but shows the same gradual decline as the season advances.

The fluctuations of the transpiration graphs give evidence again of the great variation in the daily quantity of water required to maintain normal growth, as is shown by comparing the transpiration during the period from July 21 to 23 with the adjacent three-day periods.

While the fluctuations of the different crops from day to day are similar, marked differences in the pitch of the graphs are noticeable as the season advances. The short season crops in general show a gradual increase in transpiration from seedtime to a little past the middle of the growth period and then an equally gradual decline to harvest.

The idea is often advanced that wheat and similar crops increase their water demand suddenly at or just before the time of heading. The measurements of the writers, however, lend no support to this conclusion. (See transpiration-evaporation ratios.) Aside from fluctuations due to weather the transpiration increases uniformly during this period.

WATER LOSS DURING PERIODS OF MAXIMUM TRANSPIRATION

It has already been shown in the case of annual crop plants that the transpiration rises to a maximum near the middle of the growth period and then decreases until the plants are harvested. This is especially true of grain crops. On the other hand, the transpiration of perennial forage crops, such as alfalfa, increases steadily to a maximum at the

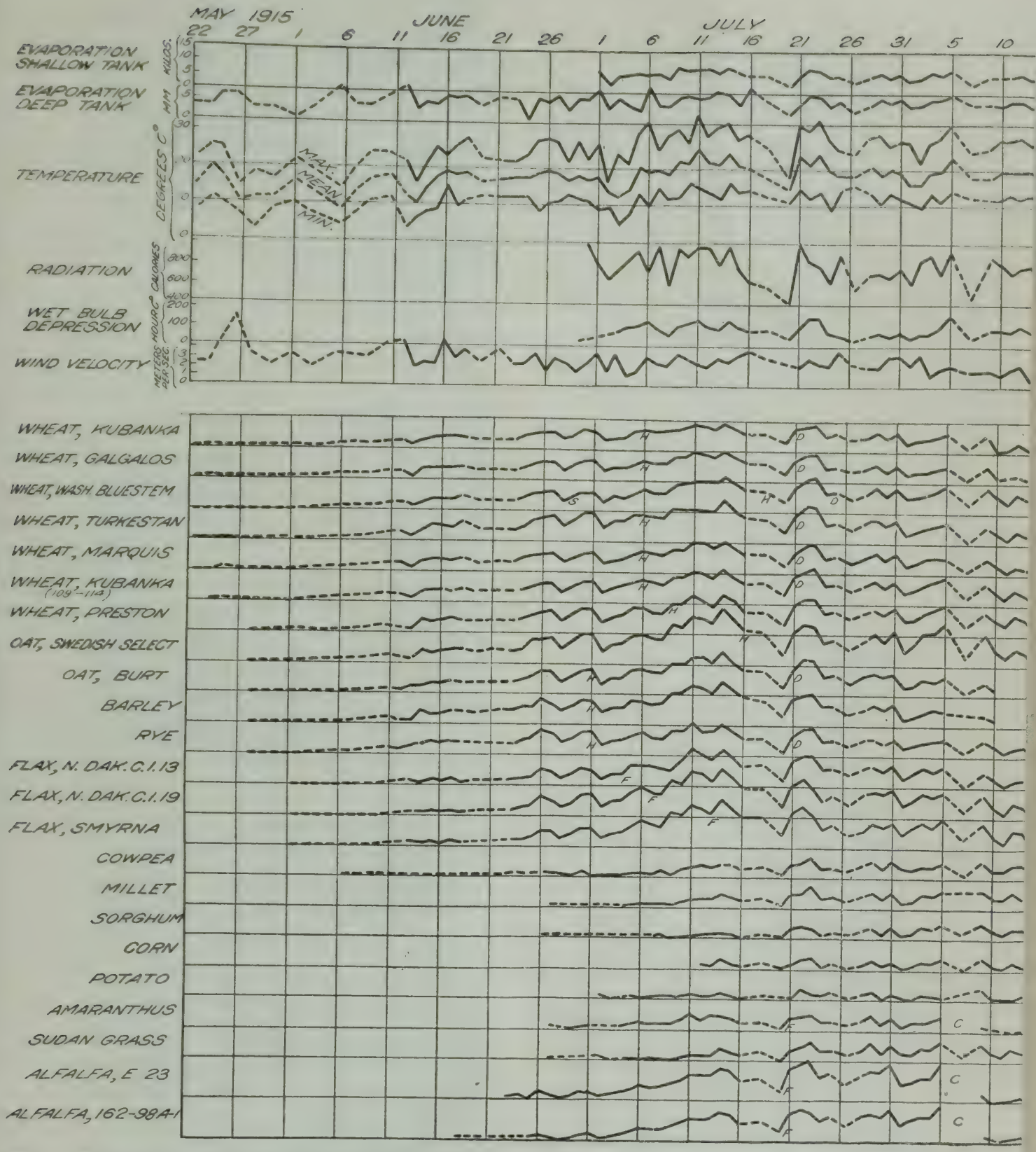

FIG. 3.—Graphs showing the daily intensity of environmental factors and the daily transpiration of 23 crops for the year 1915. The evap square meter. The evaporation from the large tank is given in millimeters. The temperatures are expressed in degrees centigra square centimeter on a surface normal to the sun's rays; the wet-bulb depression in hour degrees C.; the wind velocity in meters p space between the base (zero) lines for each crop representing 3 kgm., except for alfalfa, the base line of which is 5 kgm. below that that the crop was cut and was followed by a new growth from the established root system. *S* signifies that the plants were fo leaves dying.

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time of cutting. Various crops show their individuality by departing more or less from these types. By comparing the transpiration of two crops during any period with their total transpiration the relative demand of the crops for water during this period can be shown. Such a comparison is given in Table V for the 10-day period in 1914 from July 7 to 16, inclusive, which represents a period of maximum transpiration activity.

TABLE V.—*A comparison of transpiration during the 10-day period July 7 to 16, 1914, with total transpiration of crop*

Crop	Total transpiration.	Transpiration during 10-day period July 7–16.	
		Actual.	Percentage of total.
	Kgm.	*Kgm.*	
Wheat, Kubanka	160	40.0	25
Wheat, Galgalos	186	46.9	25
Oat, Swedish	158	43.4	27
Oat, Burt	154	40.5	26
Barley	91	22.0	24
Rye	121	30.5	25
Cowpea	112	31.7	28
Lupine	76	17.2	23
Millet, Kursk	89	23.9	27
Millet, Siberian	100	25.7	26
Corn, Northwestern Dent	112	27.5	25
Corn, Algeria	137	26.9	20
Sorghum, Minnesota Amber	130	26.8	21
Sorghum, Dakota Amber	126	26.2	21
Sudan grass (in inclosure)	110	29.0	26
Sudan grass (in open)	88	21.9	25

The relative transpiration of the small grains during this period was very nearly the same, ranging from 24 to 27 per cent of the total transpiration. This group, including the millets, shows a uniformity in this respect which is remarkable. Sudan grass, grown both outside and inside the screened inclosure, showed a relative transpiration loss during this period agreeing closely with the small grains. The same is true with Northwestern Dent corn, an early-maturing variety. Algeria corn and the two varieties of sorghum, on the other hand, show a lower relative transpiration during this period, owing to the fact that they mature later than the other crops considered.

Similar data covering a 10-day period in August are presented in Table VI. All of the crops show a lower transpiration than during the July period, except Sudan grass and Algeria corn, which had not yet begun to ripen.

Amaranthus and the alfalfas were cut the second time at the end of this period. The ratios show that nearly one-half of the total water used by the alfalfas in the production of the second crop was transpired during this 10-day period. In the case of Amaranthus the percentage was even greater.

TABLE VI.—*A comparison of transpiration during the 10-day period August 2 to 11, 1914, with total transpiration of crop*

Crop.	Total transpiration.	Transpiration during 10-day period Aug. 2–11.	
		Actual.	Percentage of total.
	Kgm.	*Kgm.*	
Cowpea	112	10.4	9
Lupine	76	12.4	16
Millet, Kursk	89	15.2	17
Millet, Siberian	100	16.5	17
Corn, Northwestern Dent	112	14.4	13
Corn, Algeria	137	27.0	20
Sorghum, Minnesota Amber	130	23.4	18
Sorghum, Dakota Amber	126	21.6	17
Sudan grass (in inclosure)	110	26.8	24
Sudan grass (in open)	88	24.4	28
Amaranthus	16	9.4	59
Alfalfa, Grimm, E23–20–52	60	28.3	47
Alfalfa, Grimm, E23	70	33.2	47
Alfalfa, Grimm, E23 (in open)	67	32.6	49
Alfalfa, Grimm, 162–98A	118	52.9	45

Similar determinations of the transpiration of different crops in 1915 during a 10-day period from July 7 to 16 are given in Table VII. The transpiration during this period ranged from 23 to 31 per cent of the total. In other words, one-fourth or more of the total water used by these crops was transpired during these 10 days. With the exception of the flax varieties the crops showing the highest transpiration during this period were those which matured earliest.

TABLE VII.—*A comparison of transpiration during the 10-day period July 7 to 16, 1915, with total transpiration of crop*

Crop.	Total transpiration.	Transpiration during 10-day period July 7–16.	
		Actual.	Percentage of total.
	Kgm.	*Kgm.*	
Wheat, Kubanka	85	20.5	24
Wheat, Galgalos	81	21.7	27
Wheat, Washington Bluestem	105	24.1	23
Wheat, Turkestan	111	29.0	26
Wheat, Marquis	88	22.6	25
Wheat, Kubanka	108	24.6	23
Wheat, Preston	126	28.6	23
Oat, Swedish Select	148	38.3	26
Oat, Burt	103	30.3	29
Barley, Hannchen	106	31.5	30
Rye, Spring	95	22.8	24
Flax, North Dakota (C. I. 13)	86	25.2	29
Flax, North Dakota (C. I. 19)	114	34.9	31
Flax, Smyrna	121	33.4	28

Corresponding determinations for a later 10-day period in 1915, July 27 to August 5, are given in Table VIII, which includes all crops in the 1915 daily transpiration measurements. The earlier maturing crops considered in the preceding table (VII) show a transpiration during this period amounting to 16 per cent of the total, compared with 26 per cent during the July period. Many of the crops were harvested soon after the termination of the July-August period, but the data given in Table VIII show that they were still transpiring actively.

TABLE VIII.—*A comparison of transpiration during the 10-day period July 27 to August 5, 1915, with total transpiration of crop*

Crop.	Total transpiration.	Transpiration during 10-day period July 27–Aug. 5.	
		Actual.	Percentage of total.
	Kilograms.	*Kilograms.*	
Wheat, Kubanka	85	12.6	15
Wheat, Galgalos	81	10.8	13
Wheat, Washington Bluestem	105	17.1	16
Wheat, Turkestan	111	15.1	14
Wheat, Marquis	88	13.2	15
Wheat, Kubanka	108	17.9	17
Wheat, Preston	126	20.4	16
Oat, Swedish Select	148	25.8	17
Wheat, Burt	103	15.2	15
Barley, Hannchen	106	13.8	13
Rye, spring	95	13.1	14
Flax, North Dakota (C. I. 13)	86	15.6	18
Flax, North Dakota (C. I. 19)	114	20.2	18
Flax, Smyrna	121	19.8	16
Cowpea	53	11.5	22
Millet	52	11.5	22
Sorghum	41	8.4	20
Corn	28	5.6	20
Potato	22	4.1	19
Amaranthus	31	9.0	29
Sudan grass	43	11.8	27
Alfalfa, E23	86	25.2	29
Alfalfa, 162–98A1	70	23.8	34

The alfalfa and amaranthus measurements given in Table VIII represent the first crops, which were harvested at the close of the period. These plants, together with Sudan grass, show a markedly higher transpiration loss than the small grains.

LOSS OF WATER DURING THE MAXIMUM TRANSPIRATION PERIOD PER UNIT OF DRY MATTER HARVESTED

MEAN DAILY TRANSPIRATION PER GRAM OF DRY MATTER HARVESTED

It is not possible in the case of the grain crops to determine directly the maximum transpiration per unit of dry matter without sacrificing the crop in order to find the dry weight. Such calculations can, however, be made upon the basis of the dry weight of the crop at maturity, which from a practical standpoint is of more importance than the former determination. Computations of this kind are presented in Tables IX to XII for the 10-day periods just considered.

Reference to Table IX will show that during the transpiration period July 7 to 16, 1914, the small grains were transpiring from 12 to 16 gm. of water per day per gram of dry matter harvested; cowpea and lupine, 19 gm.; and millet, sorghums, and corn, 6 to 9 gm. These quantities are approximately proportional to the water requirement of the crops.

TABLE IX.—*Loss of water per unit of dry matter harvested during the maximum transpiration period July 7 to 16, 1914* [a]

Crop.	Dry matter.	Mean daily transpiration, July 7–16.		Hourly transpiration during midday per gram of dry matter.	Daily loss of water per ton of dry matter per acre.
		Actual.	Per gram of dry matter.		
	Grams.	*Kilograms.*	*Grams.*	*Grams.*	*Acre-inches.*
Wheat, Kubanka	306	4.00	13.1	1.3	0.11
Wheat, Galgalos	298	4.69	15.7	1.6	.14
Oat, Swedish	264	4.34	16.4	1.6	.15
Oat, Burt	251	4.05	16.1	1.6	.14
Barley	182	2.20	12.1	1.2	.11
Rye	195	3.05	15.6	1.6	.14
Cowpea	170	3.17	18.6	1.9	.16
Lupine	90	1.72	19.1	1.9	.17
Millet, Kursk	301	2.39	7.9	.8	.07
Millet, Siberian	318	2.57	8.1	.8	.07
Corn, Northwestern Dent	304	2.75	9.0	.9	.08
Corn, Algeria	417	2.69	6.5	.7	.06
Sorghum, Minnesota Amber	457	2.68	5.9	.6	.05
Sorghum, Dakota Amber	427	2.62	6.1	.6	.05

[a] Mean daily evaporation, 12.3 kgm. per square meter.

Sudan grass during a 10-day period in August immediately preceding the first cutting showed an average transpiration loss of from 9 to 11 gm. per day per gram of dry matter harvested (Table X). Amaranthus and alfalfa were cropped for the second time at the end of this period. Amaranthus showed a transpiration loss of 17 gm. per day per gram of dry matter produced, while the alfalfas transpired from 36 to 56 gm. per day per gram of dry matter harvested. The Sudan grass and alfalfa grown outside the screened inclosure showed a transpiration from 27 to 30 per cent higher than the same crops inside the inclosure. This cor-

responds very closely with previous determinations of the influence of the inclosure on the water requirement.[1]

TABLE X.—*Loss of water per unit of dry matter harvested during the maximum transpiration period, August 2 to 11, 1914* [a]

Crop.	Dry matter.	Mean daily transpiration Aug. 2–11.		Hourly transpiration during midday per gram of dry matter.	Daily loss of water per ton of dry matter per acre.
		Actual.	Per gram of dry matter.		
	Grams.	*Kilograms.*	*Grams.*	*Grams.*	*Acre-inches.*
Sudan grass (in inclosure)	301	2.68	8.9	0.9	0.08
Sudan grass (in open)	216	2.44	11.3	1.1	.10
Amaranthus	54	.94	17.4	1.7	.15
Alfalfa, E23–20–52	66	2.83	42.9	4.3	.38
Alfalfa, E23	77	3.32	43.1	4.3	.38
Alfalfa, E23 (in open)	58	3.26	56.2	5.6	.50
Alfalfa, 162–98A	146	5.29	36.2	3.6	.32

[a] Mean daily evaporation 12 kgm. per square meter.

Reference has already been made to the fact that the water requirement in 1915 was much lower than in 1914. The daily transpiration loss of the small grains during a 10-day period from July 7 to 16, 1915, ranged from 9 to 13 gm. per gram of dry matter harvested (Table XI). Flax, which has a much higher water requirement than the small grains, showed during this period a daily loss of from 17 to 19 gm. of water per gram of dry matter harvested.

TABLE XI.—*Loss of water per unit of dry matter harvested during the maximum transpiration period July 7 to 16, 1915* [a]

Crop.	Dry matter.	Mean daily transpiration July 7–16.		Hourly transpiration during midday per gram of dry matter.	Daily loss of water per ton of dry matter per acre.
		Actual.	Per gram of dry matter.		
	Grams.	*Kilograms.*	*Grams.*	*Grams.*	*Acre-inches.*
Wheat, Kubanka	209	2.05	9.8	1.0	0.09
Wheat, Galgalos	168	2.17	12.9	1.3	.11
Wheat, Washington Bluestem	215	2.41	11.2	1.1	.10
Wheat, Turkestan	219	2.90	13.2	1.3	.12
Wheat, Marquis	208	2.26	10.9	1.1	.10
Wheat, Kubanka	267	2.46	9.2	.9	.08
Wheat, Preston	279	2.86	10.2	1.0	.09
Oat, Swedish Select	332	3.83	11.5	1.2	.10
Oat, Burt	233	3.03	13.0	1.3	.12
Barley	262	3.15	12.0	1.2	.11
Rye	203	2.28	11.2	1.1	.10
Flax, North Dakota (C. I. 13)	149	2.52	16.9	1.7	.15
Flax, North Dakota (C. I. 19)	186	3.49	18.8	1.9	.17
Flax, Smyrna	182	3.34	18.4	1.8	.16

[a] Mean daily evaporation, 7.3 kgm. per square meter.

[1] Briggs, L. J., and Shantz, H. L. Relative water requirement of plants. *In* Jour. Agr. Research, v. 3, no. 1, p. 1–64, 1 fig. pl. 1–7. 1914. Literature cited, p. 62–63.

Similar measurements for other crops during a later 10-day period in 1915 (Table XII) showed a marked reduction in the daily transpiration rate compared with the preceding year. The evaporation rate during the 1915 period was only about one-half that in 1914. The transpiration rate of the alfalfas during the 1915 period was also approximately one-half that observed in 1914.

TABLE XII.—*Loss of water per unit of dry matter harvested during the maximum transpiration period July 27 to August 5, 1915* [a]

Crop.	Dry matter.	Mean daily transpiration July 27–Aug. 5, 1915.		Hourly transpiration during midday per gram of dry matter.	Daily loss of water per ton of dry matter per acre.
		Actual.	Per gram of dry matter.		
	Grams.	*Kilograms.*	*Grams.*	*Grams.*	*Acre-inches.*
Cowpea	128	1.15	9.0	0.9	0.08
Millet	256	1.15	4.5	.5	.04
Sorghum	203	.84	4.1	.4	.04
Corn	112	.56	5.0	.5	.04
Potato	67	.41	6.1	.6	.05
Amaranthus	129	.90	7.0	.7	.06
Sudan grass	176	1.18	6.7	.7	.06
Alfalfa, E23	133	2.52	18.9	1.9	.17
Alfalfa, 162–98A1	115	2.38	20.7	2.1	.18

[a] Mean daily evaporation, 6.2 kgm. per square meter.

HOURLY TRANSPIRATION DURING MIDDAY PER GRAM OF DRY MATTER HARVESTED

An examination of the graphs of hourly transpiration on clear days [1] shows that the transpiration during one hour at or near midday in midsummer is approximately one-tenth of the total transpiration for the day. The hourly transpiration of different crops has been calculated on this basis for the midday hours during the 10-day maximum transpiration periods considered above (Tables IX to XII). In 1914 the midday transpiration of the small grains ranged from 1.2 gm. per hour per gram of dry matter for barley to 1.6 gm. for wheat, oat, and rye (Table IX). In other words, a crop of oat yielding 1 ton of dry matter per acre would have lost 1.6 tons of water per acre per hour during the midday hours of its maximum transpiration period.

Cowpea and lupine lost each hour during midday an amount of water equal to 1.9 times the dry weight of the crop (Table IX). Millet, corn, and sorghum transpired at a much slower rate than the other crops here considered, the hourly loss of sorghum being 0.6, millet 0.8, and corn 0.7 to 0.9 that of its dry weight.

[1] Briggs, L. J., and Shantz, H. L. Hourly transpiration rate on clear days as determined by cyclic environmental factors. *In* Jour. Agr. Research, v. 5, no. 14, p. 583–650, 22 fig., pl. 53–55. 1916. Literature cited, p. 648–649.

During the August transpiration period in 1914 (Table X) the alfalfas showed an hourly transpiration loss near midday of from 3.6 to 5.6 gm. per gram of dry matter. In other words, during the last third of the growth period the alfalfa crops lost during each midday hour an amount of water ranging from 3.6 to 5.6 times the dry weight of the crop.

The conditions in 1915, as has already been mentioned, were less severe than in 1914. The evaporation during the July maximum transpiration period in 1915 was only 60 per cent of that during the corresponding period in 1914. The transpiration loss of the small grains each hour during midday was approximately equal to the dry weight of the crop (Table XI). The transpiration loss of the flax varieties was nearly twice as great.

During the second transpiration period considered in 1915 (Table XII), the midday loss was still further reduced, being approximately one-half that observed in 1914.

DAILY LOSS OF WATER IN ACRE-INCHES PER TON OF DRY MATTER HARVESTED PER ACRE

From a practical standpoint it is desirable to express the daily transpiration loss in terms of acre-inches of water per ton of dry matter produced per acre. This is given in the last column of Tables IX to XII. A wheat crop yielding a ton of dry matter (grain and straw combined) would thrash approximately 12 bushels of wheat. Such a crop at Akron in 1914 would have used approximately 0.13 acre-inch of water each day during its maximum transpiration period. Millet, corn, and sorghum required approximately one-half this amount per ton of dry matter, while the alfalfas during their maximum transpiration period required the equivalent of a rainfall of from 0.3 to 0.5 inch per day per ton of dry matter produced per acre.

LOSS OF WATER DURING MAXIMUM TRANSPIRATION PERIOD PER SQUARE METER OF PLANT SURFACE

The surface area of the plant tissues of the different crops included in the transpiration measurements was determined from a selected sample of the plants in one pot of each set. The ratio of the portion selected to the whole crop was found by comparing the green weight of the sample (taken immediately after cutting) with the green weight of the whole crop. The area of the selected portion was determined by one of the following methods: (1) By direct measurement of the length and breadth (or diameter) of the leaves or stems; (2) by pasting the leaf and flower parts on surgeon's tape and determining the area by measurements; (3) by prints of the pasted leaves on squared photographic paper, the area being determined either by direct counts of the squares on the paper or by a planimeter. From the total area of the sample and

the ratio of the weights the mean surface area per pot was computed. The area measurements were made at harvest time and include the area of matured leaves as well as the fresh leaves and stems. The maximum transpiring area is therefore represented in these measurements (Tables XIII to XVI).

TABLE XIII.—*Transpiration per square meter of plant surface during the 10-day period July 7 to 16, 1914*[a]

Crop.	Plant surface.	Daily transpiration July 7–16.		Hourly transpiration per square meter of plant surface during midday.	Ratio of transpiration per square meter to evaporation per square meter.
		Actual.	Per square meter of plant surface.		
	Sq. meters.	*Kgm.*	*Kgm.*	*Gm.*	
Wheat, Kubanka	2.45	4.00	1.63	163	0.13
Wheat, Galgalos	2.68	4.69	1.75	175	.14
Oat, Swedish	3.42	4.34	1.27	127	.10
Oat, Burt	2.46	4.05	1.65	165	.13
Barley	1.95	2.20	11.3	113	.09
Rye	1.99	3.05	1.53	153	.12
Millet, Kursk	3.15	2.39	.76	76	.06
Millet, Siberian	4.20	2.57	.61	61	.05
Sorghum, Dakota Amber	1.76	2.62	1.49	149	.12

[a] Mean daily evaporation=12.3 kgm. per square meter. Hourly evaporation during midday=1,230 gm. per square meter.

TABLE XIV.—*Transpiration per square meter of plant surface during the 10-day period August 2 to 11, 1914*[a]

Crop.	Plant surface.	Daily transpiration Aug. 2–11.		Hourly transpiration per square meter of plant surface during midday.	Ratio of transpiration per square meter to evaporation per square meter.
		Actual.	Per square meter of plant surface.		
	Sq. meters.	*Kgm.*	*Kgm.*	*Gm.*	
Sudan grass (in inclosure)	3.27	2.68	0.82	82	0.07
Sudan grass (in open)	1.90	2.44	1.28	128	.11
Amaranthus	.67	.94	1.40	140	.12
Alfalfa, E23–20–52	1.71	2.83	1.66	166	.14
Alfalfa, E23	2.08	3.32	1.59	159	.13
Alfalfa, E23 (in open)	.95	3.26	3.43	343	.29
Alfalfa, 162–98A	3.37	5.29	1.57	157	.13

[a] Mean daily evaporation=12 kgm. per square meter. Hourly evaporation during midday=1,200 gm. per square meter.

TABLE XV.—*Transpiration per square meter of plant surface during the 10-day period July 7 to 16, 1915* [a]

Crop.	Plant surface.	Daily transpiration July 7–16.		Hourly transpiration per square meter of plant surface during midday.	Ratio of transpiration per square meter to evaporation per square meter.
		Actual.	Per square meter of plant surface.		
	Sq. meters.	*Kgm.*	*Kgm.*	*Gm.*	
Wheat, Kubanka	1.97	2.05	1.04	104	0.14
Wheat, Galgalos	1.47	2.17	1.48	148	.20
Wheat, Washington Bluestem	2.57	2.41	.94	94	.13
Wheat, Turkestan	2.10	2.90	1.38	138	.19
Wheat, Marquis	1.78	2.26	1.27	127	.17
Wheat, Kubanka	2.16	2.46	1.14	114	.16
Wheat, Preston	3.70	2.86	.77	77	.11
Oat, Swedish Select	2.82	3.83	1.36	136	.19
Oat, Burt	1.95	3.03	1.55	155	.21
Barley	1.74	3.15	1.81	181	.25
Rye	1.50	2.28	1.52	152	.21
Flax, North Dakota (C. I. 13)	1.49	2.52	1.69	169	.23
Flax, North Dakota (C. I. 19)	2.73	3.49	1.28	128	.18
Flax, Smyrna	3.54	3.34	.94	94	.13

[a] Mean daily evaporation=7.3 kgm. per square meter. Hourly evaporation during midday 730 gm. per square meter.

TABLE XVI.—*Transpiration per square meter of plant surface during the 10-day period July 27 to August 5, 1915* [a]

Crop.	Plant surface.	Daily transpiration July 27–Aug. 5.		Hourly transpiration per square meter of plant surface during midday.	Ratio transpiration per square meter to evaporation per square meter.
		Actual.	Per square meter of plant surface.		
	Sq. meters.	*Kgm.*	*Kgm.*	*Gm.*	
Cowpea	2.14	1.15	0.54	54	.08
Millet, Kursk	2.20	1.15	.52	52	.08
Sorghum	1.09	.84	.77	77	.12
Corn	.97	.56	.58	58	.09
Amaranthus	1.71	.90	.53	53	.09
Sudan grass	2.41	1.18	.49	49	.08
Alfalfa, E23	4.53	2.52	.56	56	.09
Alfalfa, 162–98A1	2.86	2.38	.83	83	.13

[a] Mean daily evaporation=6.2 kgm. per square meter. Hourly evaporation during midday=620 gm. per square meter.

The mean daily transpiration of the small-grain crops during the maximum transpiration period in 1914 (Table XIII) was 1.49±0.08 kgm. per square meter, and in 1915 (Table XV) 1.46±0.07 kgm. per square meter. The evaporation during the 1915 period was only 59 per cent of that observed during the corresponding period in 1914. While the transpiration periods selected were the same (July 7–16), the crops in 1914 were relatively more advanced showing evidences of ripening in some cases. This appears to be responsible, in part at least, for the observed agreement in the transpiration rate during the two years, although it is of course possible that under the weather conditions prevailing in 1915 a morphological adjustment took place, which increased the transpiration coefficient per unit area.

During the August transpiration period of 1914 the transpiration rate per square meter of plant surface (Table XIV) ranged from 81 gm. per hour for Sudan grass to 166 gm. for a variety of Grimm alfalfa, these plants being grown in the screened inclosure. The same plants grown in the open showed a transpiration loss of from 128 to 343 gm. of water per square meter of surface per hour. The water requirement of Sudan grass in the inclosure was 10 per cent below that of the crop grown in the open, while in the case of alfalfa the inclosure reduced the water requirement 22 per cent (Table II). The reduction in transpiration per unit area of the crops grown inside the inclosure is over twice that indicated from the water-requirement measurements. Here, again, there is an indication of a morphological adjustment resulting from the difference in exposure.

Periods of maximum transpiration may be due to extreme weather conditions or to the transpiration coefficient having reached its maximum or to both. It would appear that the maximum transpiration period of the small grains in 1914 was determined largely by weather conditions, since it falls rather late in the period of growth, and some of the grains had already begun to ripen. In 1915 this period corresponds more nearly to the period of maximum transpiration coefficient of the small grains. During the July period in 1914 (Table XIII) the transpiration per square meter per hour during midday ranged from 61 gm. for Siberian millet to 175 gm. for Galgalos wheat. The millets were just heading at this time, and oats, barley, and rye had begun to ripen, although the transpiration coefficient was still apparently near the maximum.

The loss of water from the plant surfaces during this period was 5 to 14 per cent of that from a water surface of equal area. In 1915 the plant surfaces lost relatively more, ranging from 10 to 25 per cent of that from a free water surface of the same area (Table XV).

It should be noted in this connection that the evaporation measurements were made in the open, while the transpiration measurements for the most part were made within the screened inclosure. The data just given regarding the transpiration of the plants relative to an equal free water surface are consequently somewhat too low.

During the August period in 1914 the transpiration measurements for alfalfa and Sudan grass were made both inside and outside the screened inclosure (Table XIV). The hourly transpiration of Sudan grass in the open was 11 per cent and that of alfalfa 29 per cent of the evaporation loss from a water surface of equal area as compared with a loss within the inclosure of 7 and 13 per cent, respectively.

The transpiration of the different plants per unit area of plant surface shows less variation than the transpiration per unit weight of dry matter produced. In other words, the greater efficiency exercised by some plants in the use of water appears to be due more to a reduction in plant surface than to a reduction of transpiration per unit area of surface. The latter effect is, however, in evidence, the plants characterized by a low-water requirement usually showing a somewhat lower transpiration rate per unit area.[1]

The determination of the surface area of a large plant from measurements on a few shoots or branches is necessarily only an approximation. For different sets of plants of the same crop the results were in satisfactory agreement. Thus, measurements of six sets of Kubanka wheat gave 49, 48, 47, 44, 52, and 49 sq. cm. of surface per gram (dry weight). The measurements were less accurate in the case of plants which branch freely, such as alfalfa. The greatest uncertainty, however, appears to be in the assumption that the various surfaces presented by a plant have the same transpiration loss per unit area.

COMPARISON OF THE ENERGY RECEIVED BY DIRECT RADIATION WITH THE ENERGY DISSIPATED BY TRANSPIRATION

If the area of the shadow thrown by a plant on a plane normal to the sun's rays is known, the direct solar radiation received by the plant in one hour, expressed in gram-calories, is equal to the product of the area of the shadow in square centimeters and the direct radiation energy in calories per square centimeter per hour.

The energy dissipated by the plant through transpiration during the same period is equal to the product of the transpiration in grams and the latent heat of vaporization (536 gm.-cal. per gram). The ratio of these two quantities represents the part played by direct sunlight in transpiration, assuming that all the radiation is absorbed. Such computations have been made for a number of plants employed in the transpiration measurements of 1914, and are presented in Table XVII. The measurements are based on the hourly transpiration and hourly radiation values at midday. For plants grown in the inclosure the radiation values have been corrected for the shade of the wire screen.

[1] The high value obtained for sorghum is due to the fact that the lower leaves were harvested early to prevent loss and were not included in the area measurements made at the time the plants were cut. These lower leaves were active during the transpiration period considered, so that the transpiring area was greater than that finally measured. The transpiration recorded per unit area is therefore too high.

TABLE XVII.—*A comparison of the energy received by direct radiation with the energy dissipated by transpiration during midday*

Period and crop.	Hourly transpiration.	Radiation (cal. per sq. cm. per hour in shelter).	Cross section of plant normal to sun's rays (area of plant shadow).	Ratio of radiation energy received to energy dissipated through transpiration.
July 7–16, 1914.	*Gm.*		*Sq. cm.*	
Wheat, Kubanka	400	58	2,000	0.54
Wheat, Galgalos	469	58	1,920	.44
Oat, Swedish Select	434	58	2,040	.51
Oat, Burt	405	58	1,940	.55
Barley	220	58	1,480	.73
Millet, Kursk	239	58	1,920	.87
Millet, Siberian	257	58	1,980	.83
Cowpea	317	58	2,230	.76
Aug. 2–11, 1914.				
Alfalfa, Grimm, E23–20–52	283	64	1,840	.78
Alfalfa, Grimm, E23	332	64	2,230	.80
Alfalfa, Grimm, E23 (in open)	326	80	2,200	1.01
Alfalfa, Grimm, 162–98A	529	64	2,870	.65

The area of the plant shadow in the case of the grain crops has been determined by considering the plants in a pot to have the form of a right cylinder, the diameter and height of which were determined from direct measurements and from photographs. The method of computing this area is readily seen from figure 4. Let x be the angle made by the sun's rays with the vertical at midday (zenith distance). The projection of the right cylinder of height h and diameter d on a plane normal to the sun's rays would give a rectangular figure with elliptical ends whose total length is $d \cos x + h \sin x$. In this expression $\frac{d}{2} \cos x$ represents the minor semidiameter of each elliptical portion, whose major semidiameter is $\frac{d}{2}$. The area of the elliptical portion is consequently $\frac{\pi}{4} d^2 \cos x$, while the area of the rectangular part of the figure is $h\, d \sin x$. The total area (a) is therefore—

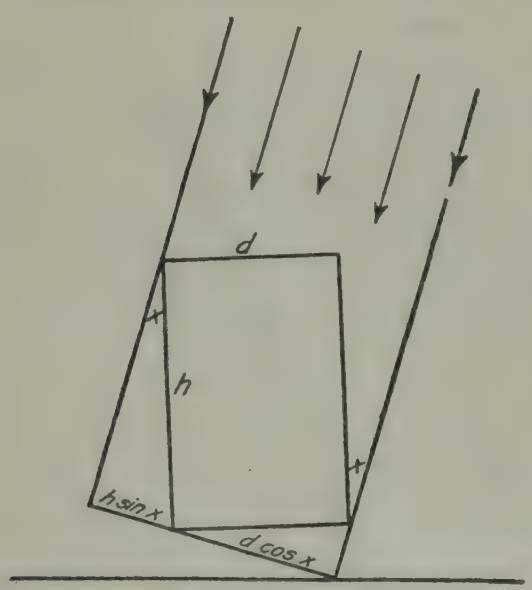

FIG. 4.—Determination of the area, on a plane normal to the sun's rays, of the shadow of a cylinder of diameter d and height h in terms of the angular departure (x) of the sun from the vertical.

$$a = \frac{\pi}{4} d^2 \cos x + h\, d \sin x.$$

The shadow areas of the other crops were computed from the projection of the geometrical figures which they most closely approximated, such as cylinders, spheres, or inverted truncated cones.

While measurements of this kind are at best approximations, the results given in Table XVII are consistent in showing that the energy received directly from the sun is insufficient to account for the energy dissipated by the plants through transpiration during the midday hours. Only in the case of alfalfa in the open is the direct solar radiation intercepted by the plant sufficient to account for the observed transpiration. Even on bright days, therefore, other sources of energy, such as the indirect radiation from the sky and from surrounding objects and the heat energy received directly from the air, contribute materially to the energy dissipated through transpiration. Comparative transpiration measurements of shaded and unshaded plants also show that the energy consumed in transpiration is only partially attributable to direct radiation.

RELATION OF TRANSPIRATION TO THE WEATHER

If transpiration is determined absolutely by the intensity of any one of the weather factors, the ratio of the daily transpiration to the daily intensity of this factor should give a regular graph when plotted. This graph, if the correlation were perfect, would be an expression of the relative transpiration coefficient of the plant considered.

In figure 5 are plotted the ratios of the daily transpiration of Kubanka wheat to the daily evaporation from the shallow tank. This graph shows a gradual increase in the transpiration coefficient to a maximum on July 13, followed by a somewhat more rapid decrease to harvest. While the graph shows many irregularities a similar response in transpiration and evaporation to changes in weather is indicated.

The second graph shows the ratio of transpiration to wet-bulb depression. Outstanding points in this graph indicate, of course, that on certain days the transpiration values were influenced by some factor other than the dryness of the air. Reference to the radiation graph will show that on such days the transpiration-radiation ratio is normal or shows a departure in the opposite sense to the ratio of transpiration to wet-bulb depression. In other words, both factors enter into the determination of the transpiration.

Similar departures are in evidence in the transpiration-temperature graph, and it will be noted that such departures are usually in an opposite sense to one or the other of the graphs just discussed. Temperature, therefore, also enters into the determination of the transpiration.

The ratio of transpiration to wind velocity is plotted at the bottom of figure 5. The change in the transpiration coefficient is evident in this graph, but no marked correlation is indicated.

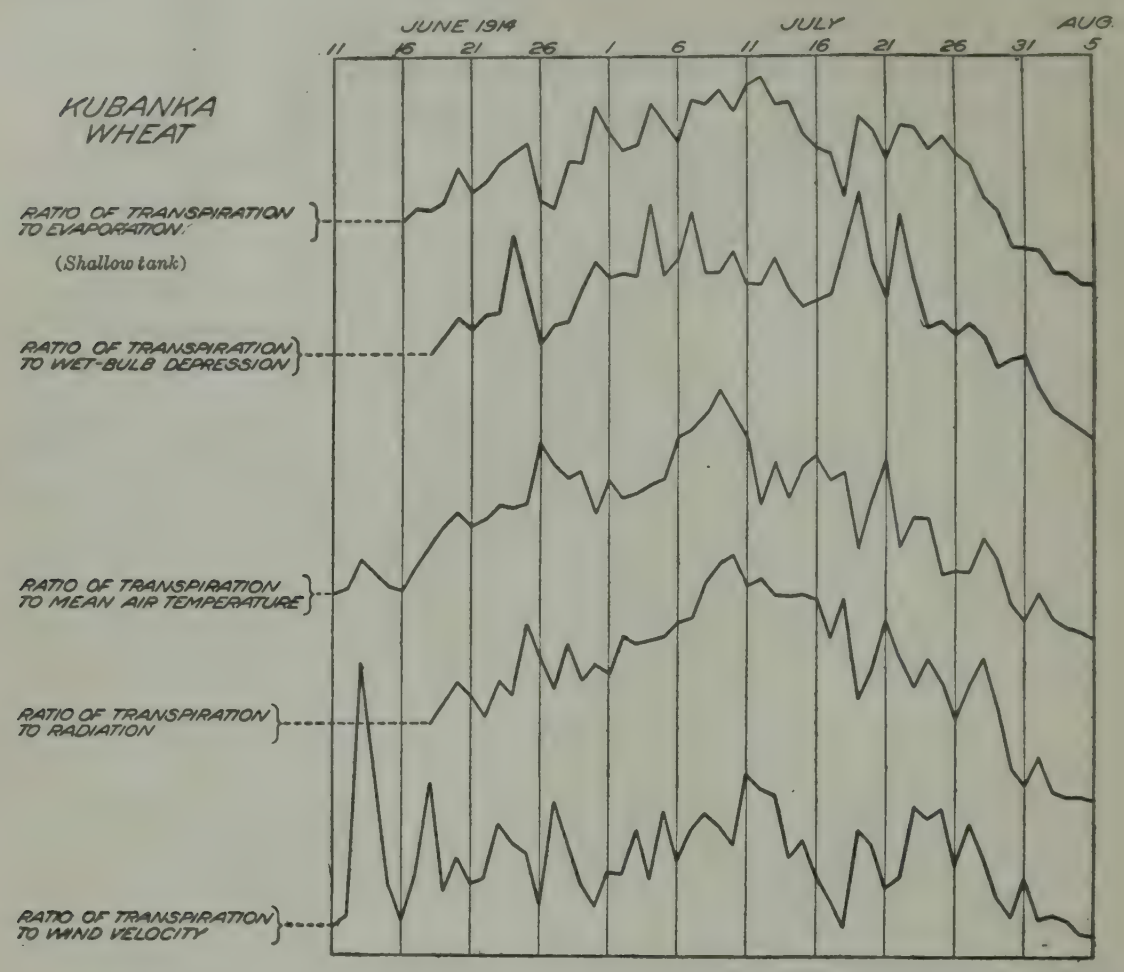
JUNE 1914
JULY
AUG.
11 16 21 26 1 6 11 16 21 26 31 5
KUBANKA WHEAT
RATIO OF TRANSPIRATION TO EVAPORATION
(Shallow tank)
RATIO OF TRANSPIRATION TO WET-BULB DEPRESSION
RATIO OF TRANSPIRATION TO MEAN AIR TEMPERATURE
RATIO OF TRANSPIRATION TO RADIATION
RATIO OF TRANSPIRATION TO WIND VELOCITY

FIG. 5.—Ratios of the daily transpiration of Kubanka wheat to the daily intensity of various weather factors, plotted with approximately the same amplitude.

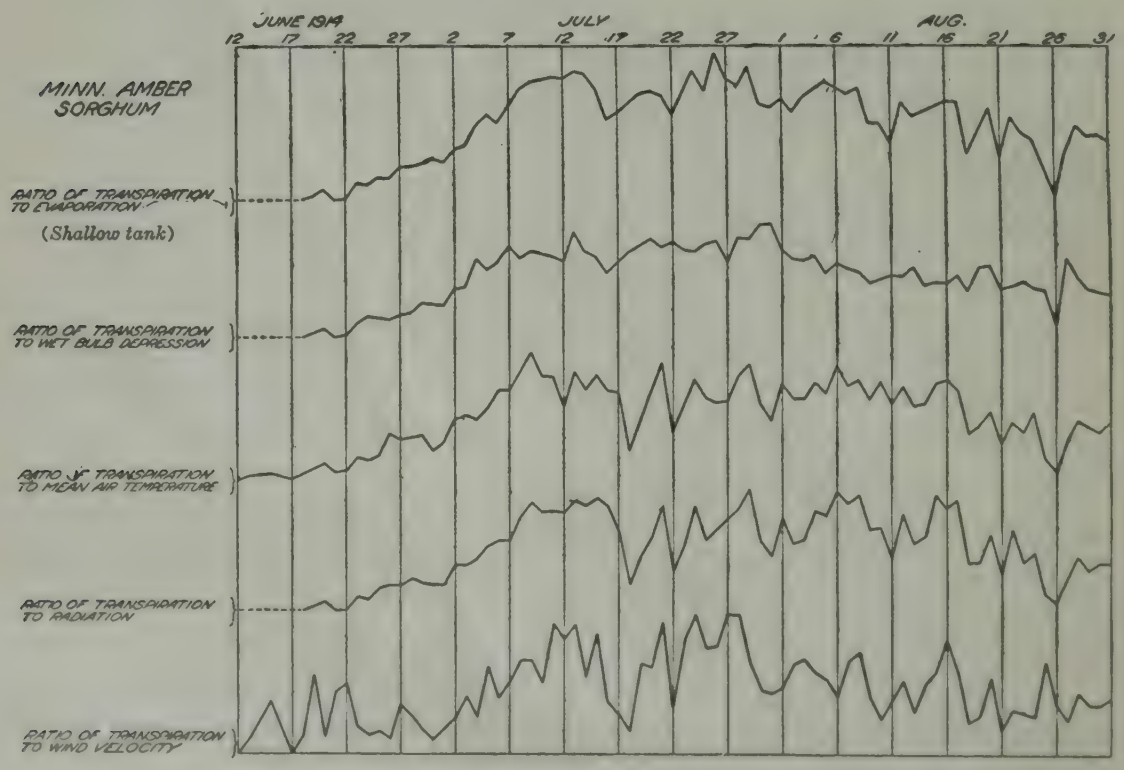
JUNE 1914
JULY
AUG.
12 17 22 27 2 7 12 17 22 27 1 6 11 16 21 26 31
MINN. AMBER SORGHUM
RATIO OF TRANSPIRATION TO EVAPORATION
(Shallow tank)
RATIO OF TRANSPIRATION TO WET BULB DEPRESSION
RATIO OF TRANSPIRATION TO MEAN AIR TEMPERATURE
RATIO OF TRANSPIRATION TO RADIATION
RATIO OF TRANSPIRATION TO WIND VELOCITY

FIG. 6.—Ratios of the daily transpiration of Minnesota Amber sorghum to the daily intensity of various weather factors, plotted with approximately the same amplitude.

An inspection of the ratio graphs of sorghum and alfalfa (figs. 6 and 7) shows, as in the case of Kubanka wheat, a dependence of transpiration upon radiation, wet-bulb depression, and temperature. A marked departure in any one of the ratio graphs is usually, though not always, accompanied by a departure in the opposite sense in one of the other graphs. The correlation of transpiration with wind velocity is low in the case of both of these crops.

MARCH OF TRANSPIRATION DURING THE GROWTH PERIOD AS SHOWN BY THE RATIO OF DAILY TRANSPIRATION TO DAILY EVAPORATION

Although the association of transpiration with wet-bulb depression is fully as marked as the transpiration-evaporation association (see correlation coefficients, p. 204), the writers have decided to employ the latter ratio to represent the change in the transpiration coefficient of the different plants, since it is already used extensively.[1]

The ratios of the daily transpiration of each crop to the daily evaporation (shallow tank) are plotted as percentages of the maximum in figures 8 and 9. By this method different crops may be easily compared.

The graphs show irregularities due to the lack of an exact correlation between evaporation and transpiration, or to errors in the measurements. On days when rain occurred the outstanding points are probably due to inaccurate determinations of the transpiration.[2] The outstanding ratios on days without rain are not explainable on this basis and are to be regarded as expressions of the inexact correlation of transpiration and evaporation as here measured.

The general trend of the graphs indicates a gradual increase in the transpiration coefficient from seedtime to a maximum which in the case of annual plants comes just before they begin to ripen.

MEASUREMENTS IN 1914

In 1914 (fig. 8) the small grains were well advanced before the transpiration measurements were begun. They did not reach their maximum transpiration rate, however, until about one month later.

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[1] Livingston, B. E. The relation of desert plants to soil moisture and to evaporation. 78 p., illus. Washington, D. C., 1906 (Carnegie Inst. Washington Pub. 50.) Literature cited, p. 77–78.

—— The resistance offered by leaves to transpirational water loss. *In* Plant World, v. 16, no. 1, p. 1–35, illus. 1913.

[2] Even a small amount of rain wets the plant thoroughly, a part of the water remaining on the surface of the plants and a part being absorbed by the dry or living leaves or caught in the leaf sheaths or flower heads. Water is also held on the surface of the pot and a small amount may find its way into the pot by suction due to the change in temperature. If the morning weighing following a rain during the night is taken as the basis of determining the transportation, on the subsequent day the transpiration is too high since some of the water is merely evaporated from the surface of the plant and pot. If the two days are combined and morning weighing discarded, the transpiration is too low since transpiration from wet plants is lower than from dry plants, and since an equivalent of the water which was absorbed must be transpired before a loss in weight can be recorded. Notwithstanding the errors of the second method, it seems best not to introduce the greater uncertainty involved in the first method and to regard the outstanding determinations on rainy days as experimental errors which can not at present be successfully overcome without actually protecting the plants from rain, which would change the conditions under which field crops are grown.

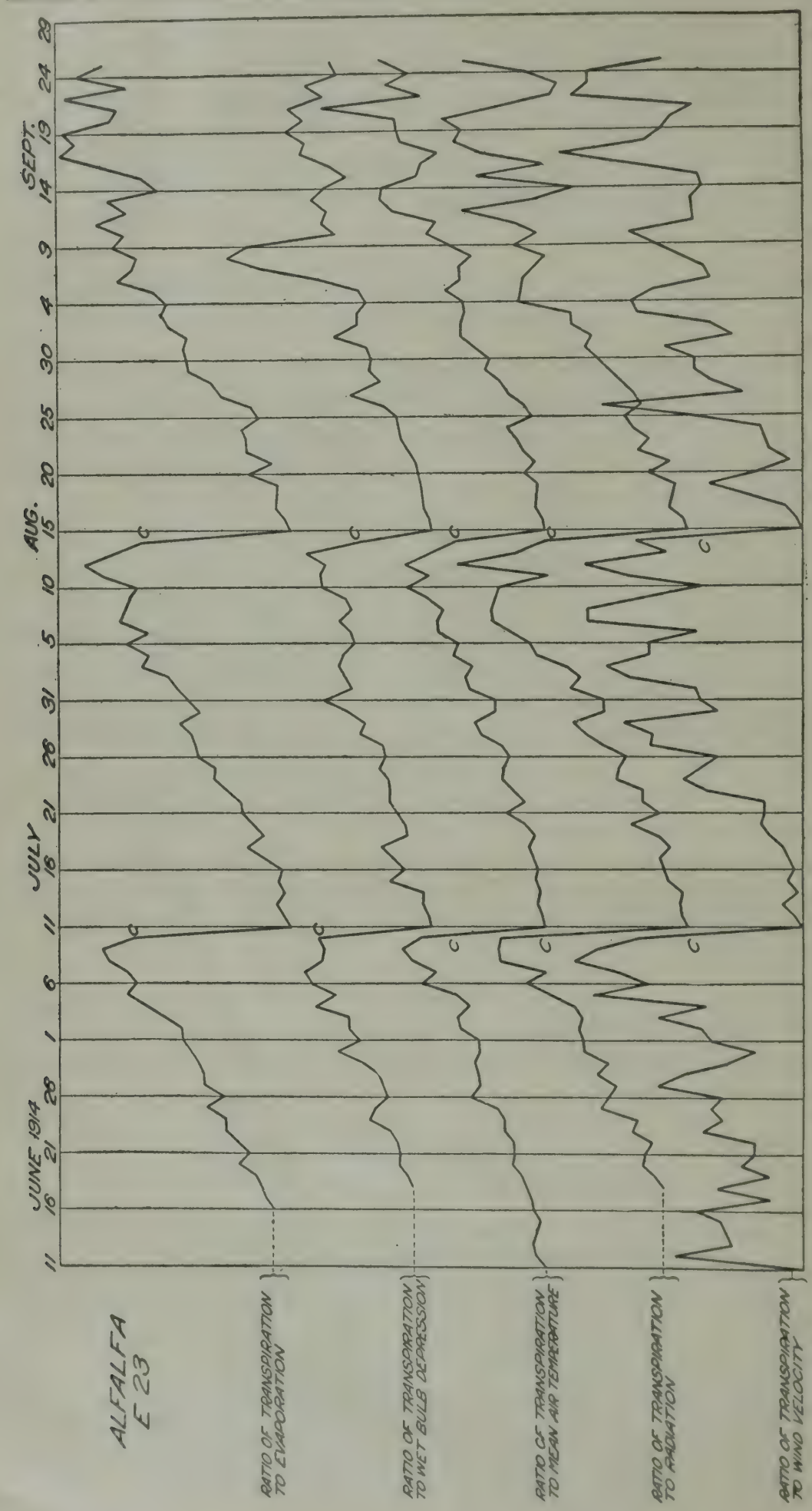


FIG. 7.—Ratios of the daily transpiration of alfalfa, E-23, to the daily intensity of various weather factors, plotted with approximately the same amplitude. *C* indicates the date of cutting.

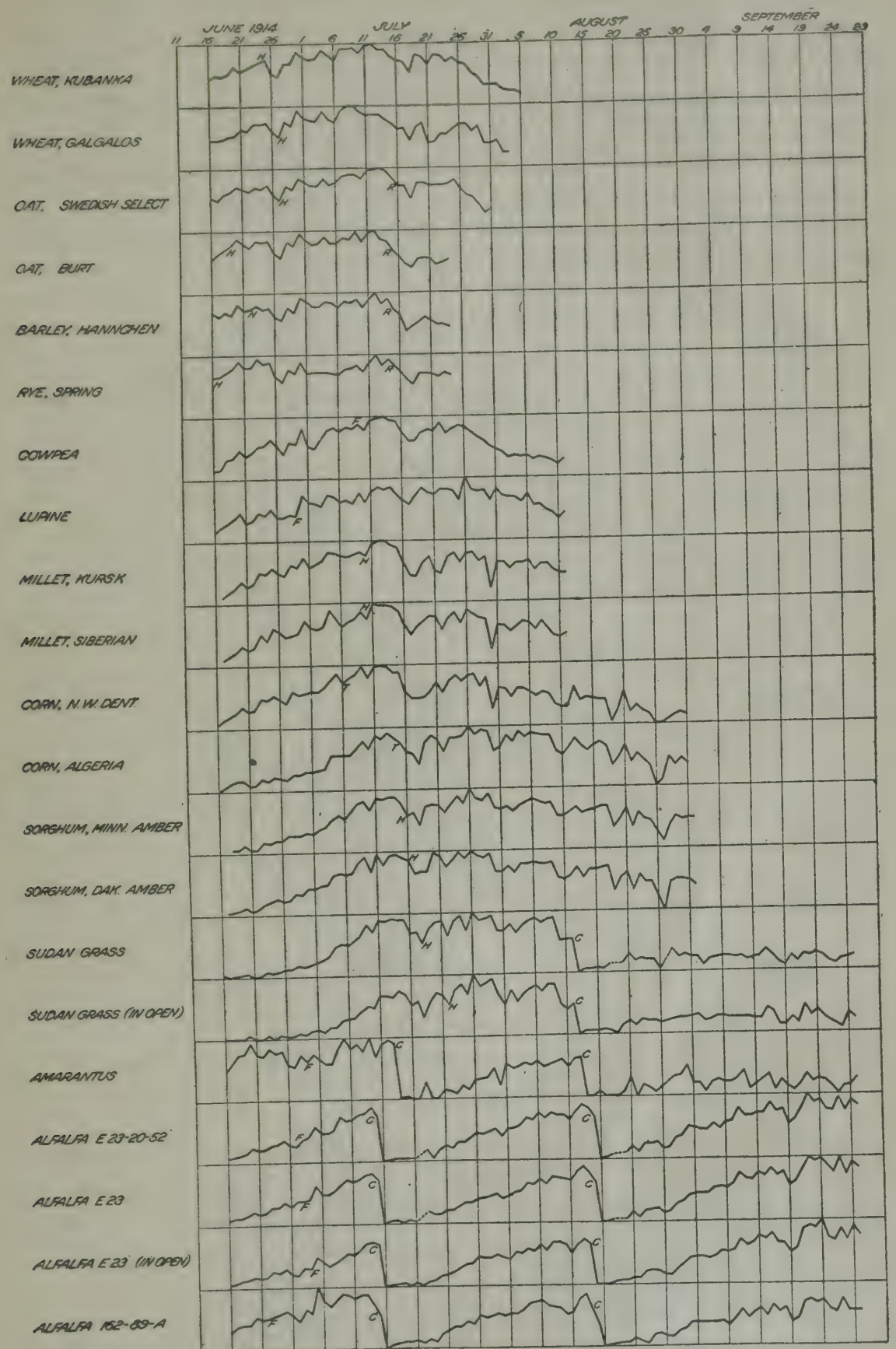


FIG. 8.—The ratio of daily transpiration of different crops in 1914 to daily evaporation (shallow tank) plotted in percentage of the maximum. The letter *C* in the graph indicates date of cutting, followed by a new growth from the established root system. *H* signifies heading; *F*, flowering; *R*, ripening; and *T*, tasseling.

The transpiration coefficient of Kubanka wheat had reached half the maximum value when the daily weighings were begun, although the total transpiration from seedtime to this date was only 10 per cent of that for the whole season. A gradual increase in the transpiration coefficient is recorded from June 16 to a maximum on July 12. Although the plants began to head on June 15, this produced no marked change in the transpiration coefficient. The drop in the graph following the period of heading is not significant in this connection since a similar drop was recorded for the other crops, and it is evidently due to the failure of

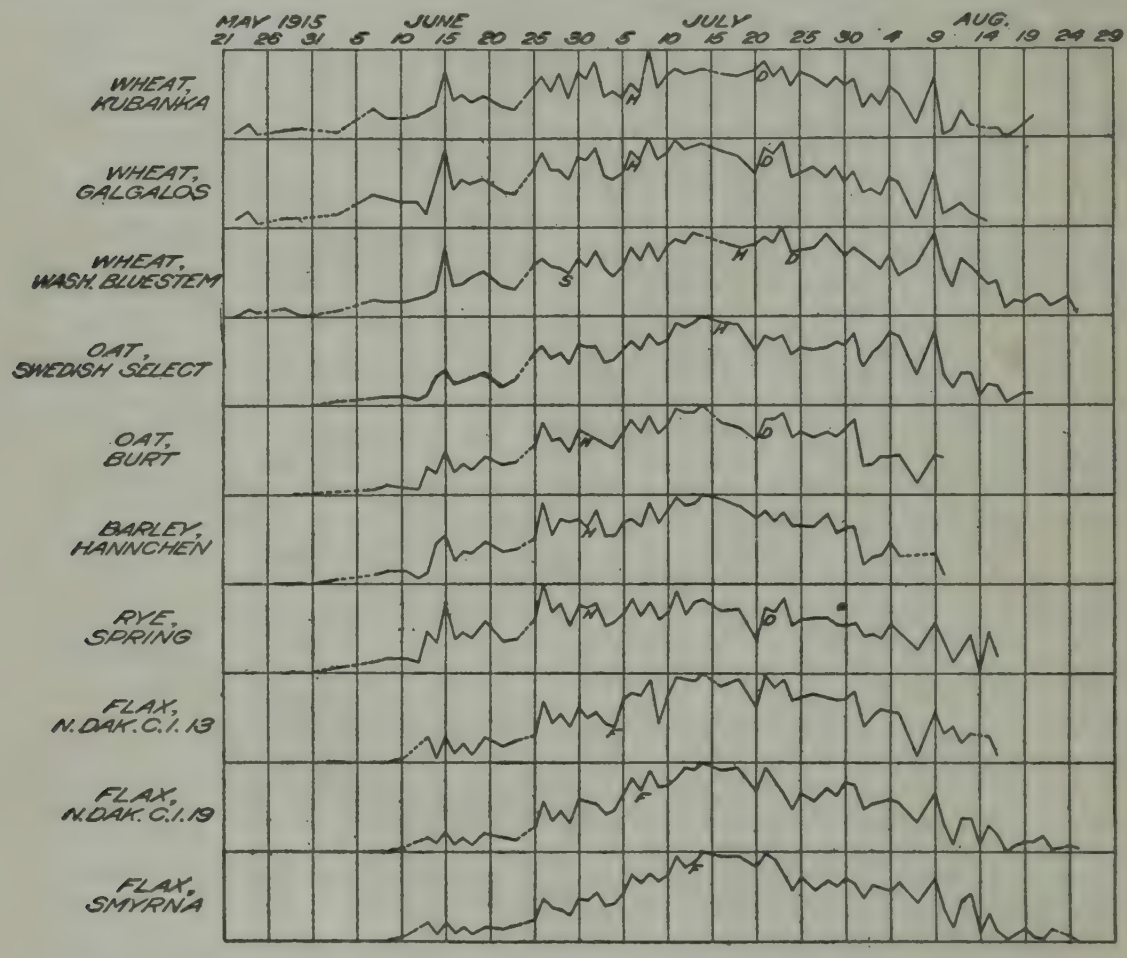


FIG. 9.—The ratio of daily transpiration of different crops grown in 1915 to daily evaporation (shallow tank) plotted in percentage of the maximum. *S* signifies that the plants were forming shoots; *H*, heading; *F*, flowering; and *D*, lower leaves dying.

evaporation and transpiration to respond proportionately to the weather conditions on these days. The gradual decrease in the transpiration coefficient following the maximum is indicated by the graph and begins almost one month before harvest. At harvest time the transpiration coefficient of Kubanka was still 20 per cent of the maximum.

Galgalos wheat which began to head two days later than Kubanka reached its maximum four days earlier. The period of gradual decline was approximately one month long and the transpiration coefficient at harvest was 20 per cent of the maximum. The depression in the curve in the early part of the period of decline is probably not significant since

it is recorded for all crops and is to be attributed largely to the errors in measuring transpiration during a period of rainy weather.

The transpiration coefficient of Swedish Select oat, which began to head on June 28, did not reach its maximum until July 13, after which it declined rather rapidly to about 25 per cent of the maximum at harvest time. Burt oat began to head much earlier, but showed the same gradual increase to the maximum. The value at harvest time was very high, amounting to 50 per cent of the maximum. Hannchen barley and Burt oat showed very little difference in the march of the transpiration coefficient.

Rye was beginning to head when the measurements were started. The transpiration coefficient was unusually uniform throughout the season. At harvest it was 65 per cent of the maximum and did not differ materially from that at the period of heading.

The graph for cowpea is almost symmetrical, beginning and ending at about 20 per cent of the maximum, which occurred just after the flowering period.

Lupine blossomed earlier than cowpea and showed no marked maximum period, the rate remaining about the same from the 10th of July to the 1st of August.

Kursk and Siberian millet showed no significant differences. The graphs include practically the whole growth period. The increase was rapid and uniform and the maximum was reached at the period of heading. The transpiration coefficient was about 40 per cent of the maximum at the time of harvest.

The two varieties of Amber sorghum showed no marked differences. Dakota Amber headed a little earlier than Minnesota Amber. The maximum transpiration coefficient of both crops occurred about 10 days after the plants began to head. The decline was gradual, the value at harvest being over 40 per cent of the maximum, notwithstanding the fact that the seeds were ripe at that time.

Sudan grass was grown in the screened inclosure and also in the open. The transpiration coefficient of the plants in the inclosure increased somewhat more rapidly at first than in the open, although the maximum was reached at the same time. The second crop was much smaller than the first and the transpiration coefficient reached only about one-fourth the value attained during the first crop.

Algeria corn reached its maximum much later than Northwestern Dent and showed no marked decrease in its transpiration coefficient, the value at harvest being 40 per cent of the maximum. Algeria corn did not ripen at Akron, which accounts for the high transpiration coefficient when the crop was harvested.

The graph for Amaranthus shows no marked change in the transpiration coefficient during the first crop. In other words, the young large-

leaved plants lost water almost as rapidly as the older plants on which the lower leaves either drop off or become relatively inactive.

Four sets of alfalfa were included in these measurements. Three of these sets were grown from seed, while the fourth alfalfa, 162–98A, was grown from cuttings. The transpiration coefficient of the latter was already 25 per cent of the maximum when the measurements were begun. Some of the plants in the first crop began to bloom when the transpiration coefficient was only 40 per cent of the maximum. In the second and third crops the plants were harvested shortly after they had developed flowers. The march of the transpiration coefficient for each variety was approximately the same, with the exception of the first crop of alfalfa, 163–98A, which developed much more rapidly than the other varieties. The set in the open developed more slowly than in the inclosure.

The relative loss of water at different periods in the growth of a crop, the evaporation rate being uniform, can readily be determined from the graphs representing the transpiration–evaporation ratio. The weekly loss from different crops expressed in percentages of the total is given in Table XVIII.

TABLE XVIII.—*Weekly transpiration under uniform conditions of evaporation expressed in terms of percentage of the total, 1914*

Crop.	Week of growth.										
	1st.	2d.	3d.	4th.	5th.	6th.	7th.	8th.	9th.	10th.	11th.
Northwestern Dent corn	.....	2	4	10	13	12	14	14	13	11	7
Algeria corn	.....	2	4	8	13	12	14	15	13	11	8
Sorghum, Minnesota Amber	.....	2	5	11	14	12	15	13	11	10	7
Sorghum, Dakota Amber	.....	2	5	10	14	14	15	12	11	10	7
Sudan grass (first crop)	.....	1	2	8	17	17	18	19	16	.....	.....
Sudan grass (first crop in open)	.....	1	1	6	17	17	20	20	18	.....	.....
Alfalfa, E23–20–52 (second crop)	2	10	22	30	36	.....	.....	.....	.....	.....	.....
Alfalfa, E23 (second crop)	3	10	21	30	36	.....	.....	.....	.....	.....	.....
Alfalfa, E23 (second crop in open)	2	11	23	32	32	.....	.....	.....	.....	.....	.....
Alfalfa, 162–98A (second crop)	4	13	23	30	30	.....	.....	.....	.....	.....	.....

The loss from corn and sorghum during the week preceding the measurements was very slight, amounting to less than 1 per cent of the total. The mean transpiration of the four crops for the second and succeeding weeks was 2, 5, 10, 14, 13, 15, 14, 12, 11, and 7 per cent of the total. The drop in the sixth week is not significant and is probably due to imperfect measurements of transpiration during days when rain occurred.

Sudan grass in its early stages of growth developed more rapidly in the inclosure than in the open. Alfalfa produced its second crop in a period of five weeks, two-thirds of the total transpiration occurring during the last two weeks of the growth period. The transpiration coefficient changed very rapidly, being approximately one-third the maximum in the second week and two-thirds the maximum in the third week.

MEASUREMENTS IN 1915

The daily weighings in 1915 were begun as soon as the plants had started to grow, and the total transpiration period of wheat, oats, barley, rye, and flax is included in the transpiration-evaporation graphs presented in figure 9. These graphs show the march in the transpiration independently of the fluctuations due to daily changes in the weather, assuming the latter to be represented by the fluctuations in evaporation. On the same assumption, the march of the weekly transpiration under uniform weather conditions is expressed numerically in Table XIX in per cent of the total transpiration.

TABLE XIX.—*Weekly transpiration under uniform conditions of evaporation expressed in terms of percentage of the total, 1915*

Crop.	Week of growth.												
	1st.	2d.	3d.	4th.	5th.	6th.	7th.	8th.	9th.	10th.	11th.	12th.	13th.
Wheat, Kubanka 1440	1	2	4	8	9	12	12	13	13	7	8	4	2
Wheat, Galgalos	2	3	5	8	9	11	13	15	12	10	8	4	.....
Wheat, Washington Bluestem	1	1	3	6	7	9	11	14	14	12	10	9	4
Oat, Swedish Select	1	2	6	8	11	14	18	13	11	11	5	.....	.....
Oat, Burt	0	3	7	10	12	16	19	14	12	7	.....	.....	.....
Barley, Hannchen	1	2	8	10	14	16	18	15	10	6	.....	.....	.....
Rye, spring	1	4	8	11	12	13	14	12	10	8	7	.....	.....
Flax, North Dakota (C. I. 13)	2	4	8	10	17	17	16	13	8	5	.....	.....	.....
Flax, North Dakota (C. I. 19)	2	3	6	10	18	18	14	13	10	4	2	.....	.....
Flax, Smyrna	2	3	6	9	15	19	15	12	12	5	2	.....	.....

The wheats reached their maximum transpiration in the eighth week following emergence and were harvested in the twelfth or thirteenth week. Oats, barley, and rye, which were planted a little later, reached their maximum in the seventh week of growth and were harvested in the tenth or eleventh week. The flax varieties reached their maximum in the sixth week, although they were not harvested until five weeks later.

THE TRANSPIRATION COEFFICIENT DURING THE EARLY PERIODS OF GROWTH

The transpiration-evaporation graphs during the early development of the crops either approximate a straight line, or curve upward as in the case of Sudan grass. The latter form suggests an exponential relationship, which would mean that the rate of increase in the transpiration coefficient is proportional to the transpiration coefficient itself.

Let T represent the transpiration, E the evaporation, and $\frac{T}{E}=k$ the transpiration coefficient (referred to evaporation) at the time t in the development of the crop. If the rate of increase of the transpiration coefficient $\frac{dk}{dt}$ is proportional to the transpiration coefficient, then

$$\frac{dk}{dt}=a'k \qquad (1)$$

in which a' is a constant of proportionality.

Integrating equation (1) and transforming to common logarithms, we have

$$\log_{10} k = at + c \tag{2}$$

in which c is the logarithm of k when $t = o$—that is, at the beginning of the period under discussion. Expressing (2) exponentially, we have

$$k = 10^{at+c} = k_0 . 10^{at} \tag{3}$$

Therefore if the logarithms of the daily transpiration when plotted against the time form a straight line, the condition expressed in equation (2) is satisfied, and the transpiration coefficient increases in accordance with the assumption made above.

The accompanying graphs (fig. 10 to 15) show that an approximate linear relationship does exist between the logarithm of the transpiration coefficient and the time in the case of corn, sorghum, Sudan grass,

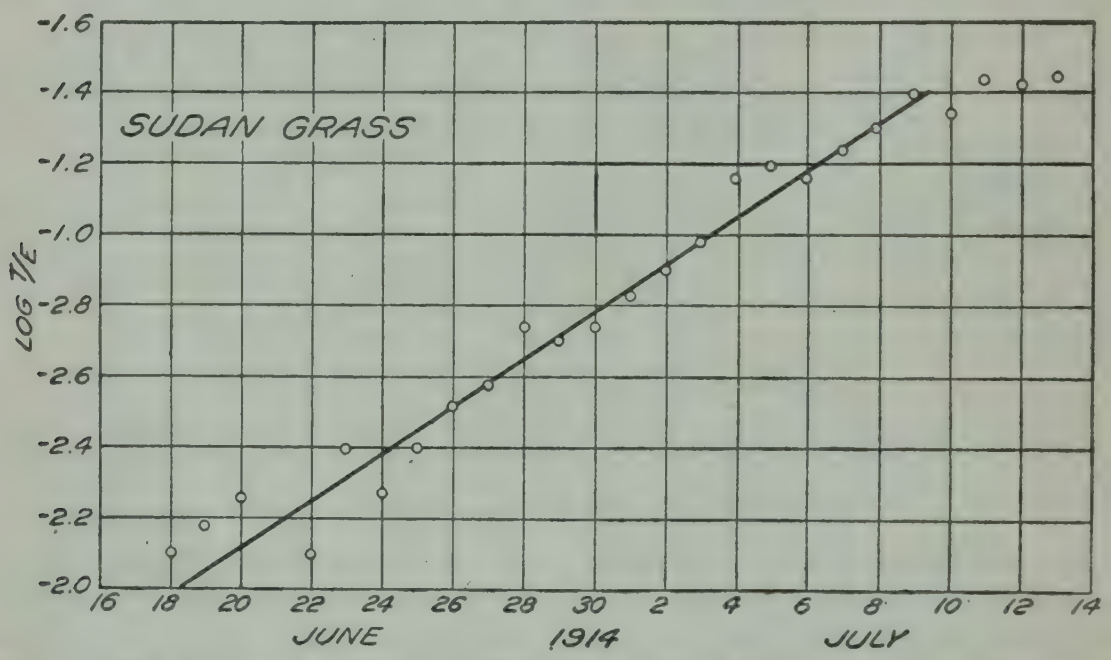


FIG. 10.—Graph showing a linear relation between the logarithm of the transpiration-evaporation ratio of Sudan grass and the time.

and alfalfa. The transpiration coefficient of these plants during the early stages of growth therefore changes exponentially.

The numerical value of the coefficient a may now be computed. This is represented by the slope of the graphs in figures 10 to 15 and from equation (2) it follows that

$$a = \frac{\log k_t - \log k_o}{t} \tag{4}$$

The significance of a can be readily seen by a comparison of equation (3) with the compound interest law

$$k = k_o \left(1 + \frac{r}{100}\right) t,$$

from which it is evident that

$$10^a = 1 + \frac{r}{100}, \text{ or}$$

$$a = \log_{10}\left(1 + \frac{r}{100}\right),$$

in which $\frac{r}{100}$ is the interest rate. From this relationship the daily rate of increase (daily interest) can be readily determined.

The rate of increase may also be expressed in terms of the time required for k to double in value.

Let k_1 be the value of k at the time t_1. If $k = 2k_1$,

$$\frac{k}{k_1} = \frac{10^{at}}{10^{at_1}} = 10^{a(t - t_1)} = 2,$$

$$a(t - t_1) = \log_{10} 2 = 0.3010,$$

$$t - t_1 = \frac{0.3010}{a}$$

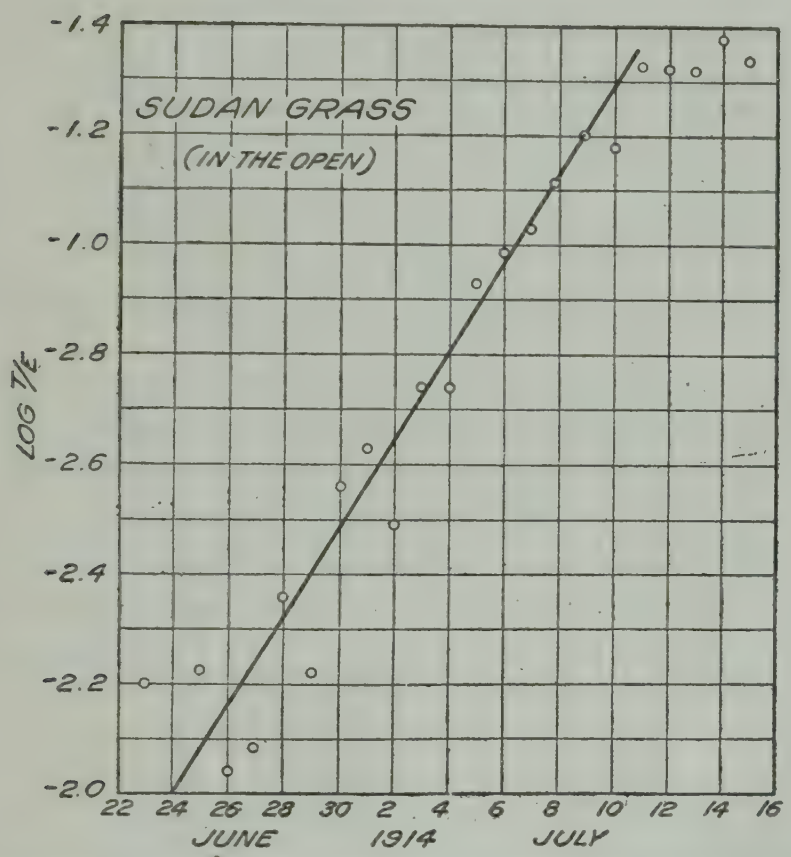


FIG. 11.—Graph showing a linear relation between the logarithm of the transpiration-evaporation ratio of Sudan grass (grown in the open) and the time.

The values of the coefficient a, the rate of increase r, and the time $(t - t_1)$ required for k to double in value are given in Table XX for the various crops considered in figures 10 to 15.

It will be seen from Table XX that the daily increase in the transpiration coefficient of Sudan grass during the early stages of growth was extremely rapid, ranging from 16 to 21 per cent. Since this is compounded daily, four days are required for the transpiration coefficient to double in value during this active growth period. The growth rate during the early stages of Algeria corn and sorghum was less rapid, about seven days being required for k to double in value.

The results obtained in case of alfalfa are of special interest, for the plants were much more advanced than those just considered. In fact, in two of the three alfalfa measurements given the periods were terminated by the harvesting of the plants while in full bloom. The data show that during the periods considered the transpiration coefficient of alfalfa was compounding at the rate of 8 to 9 per cent per day, thus doubling in value every 8 or 9 days.

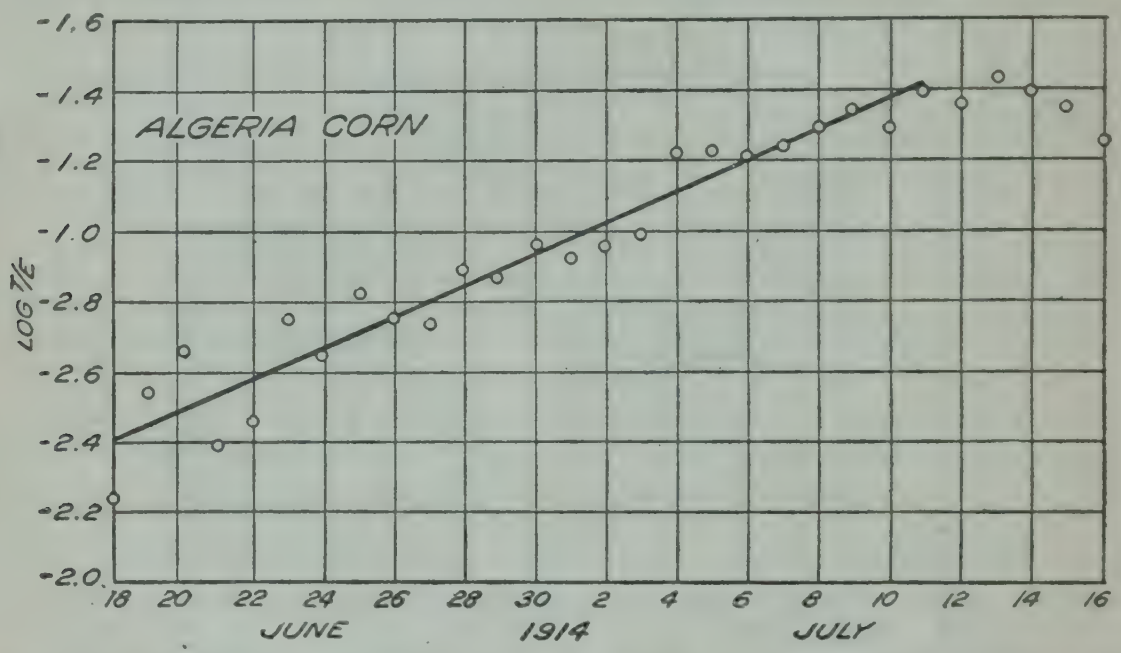


FIG. 12.—Graph showing a linear relation between the logarithm of the transpiration-evaporation ratio of Algeria corn and the time.

TABLE XX.—*Rate of increase in the transpiration coefficient of different crops in 1914*

Crop.	Observation period.	(*a*)	Daily rate of increase.	Days required for *k* to double in value.
			Per cent.	
Corn, Northwestern Dent	June 18–July 9	0.026	6.2	11.6
Corn, Algeria	June 18–July 11	.044	10.7	6.8
Sorghum, Minnesota Amber	June 18–July 9	.041	9.9	7.3
Sudan grass (in inclosure)	June 18–July 10	.066	16.4	4.6
Sudan grass (in open)	June 24–July 11	.082	20.8	3.7
Alfalfa, E23 (first crop in open)	June 16–July 10	.033	7.9	9.1
Alfalfa, E23–20–52 (first crop)	June 19–July 9	.037	8.9	8.1
Alfalfa, E23–20–52 (second crop)	July 18–Aug. 5	.037	8.9	8.1

A test of the validity of the computations may be obtained by comparing the observed graph of the transpiration coefficient with that computed from equation (3). The result of such a computation in the case of Sudan grass (in the open) is given in figure 16, the computed graph being represented by the smooth curve, while the observed values are represented by circles.

RELATIVE DAILY TRANSPIRATION OF DIFFERENT CROPS

In the preceding discussion the transpiration-evaporation ratio has been used in order to eliminate, so far as possible, the daily fluctuations

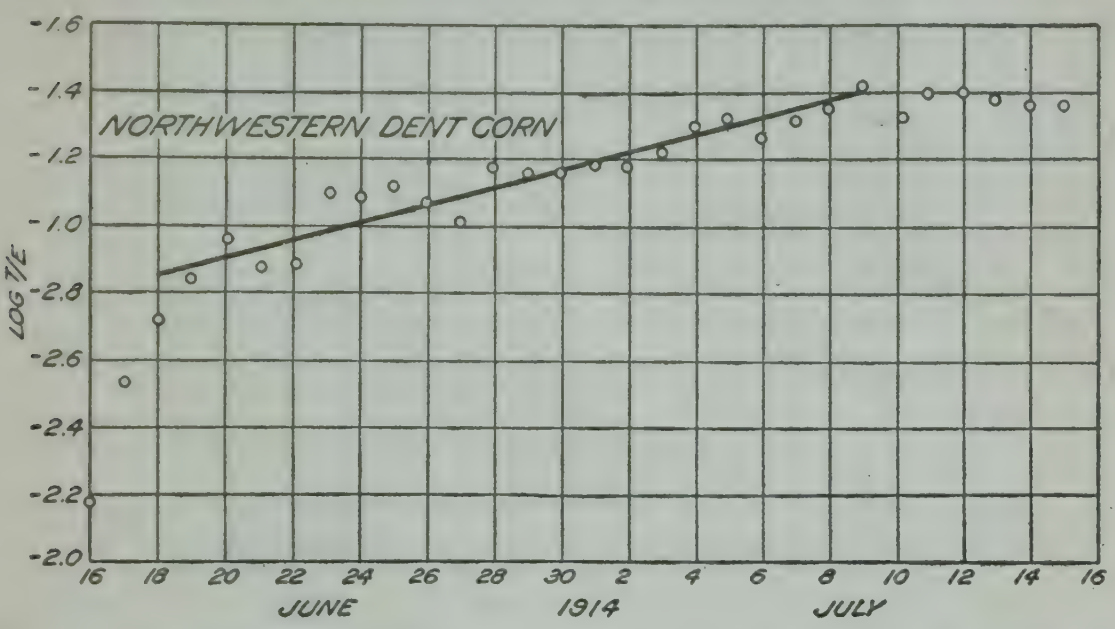


FIG. 13.—Graph showing a linear relation between the logarithm of the transpiration-evaporation ratio of Northwestern Dent corn and the time.

in transpiration due to changes in weather. The relative march of the transpiration of any two crops can, however, be determined directly

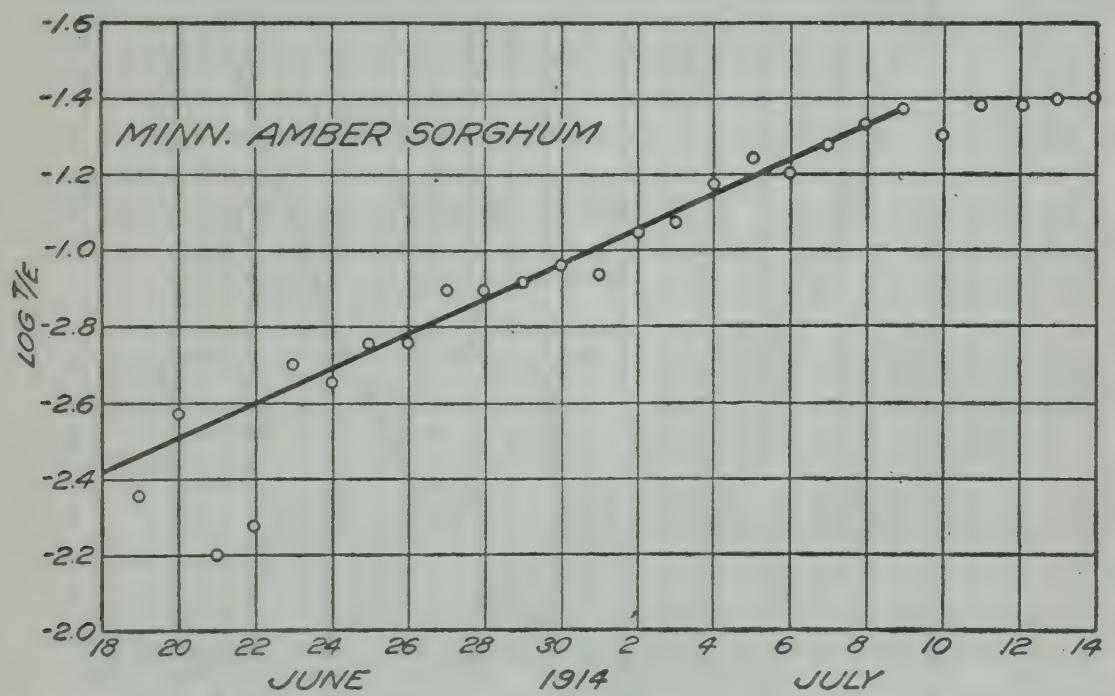


FIG. 14.—Graph showing a linear relation between the logarithm of the transpiration-evaporation ratio of Minnesota Amber sorghum and the time.

by comparing the transpiration day by day; in other words, if two crops are grown simultaneously and have approximately the same length of growing season, the relative change in transpiration may be obtained by

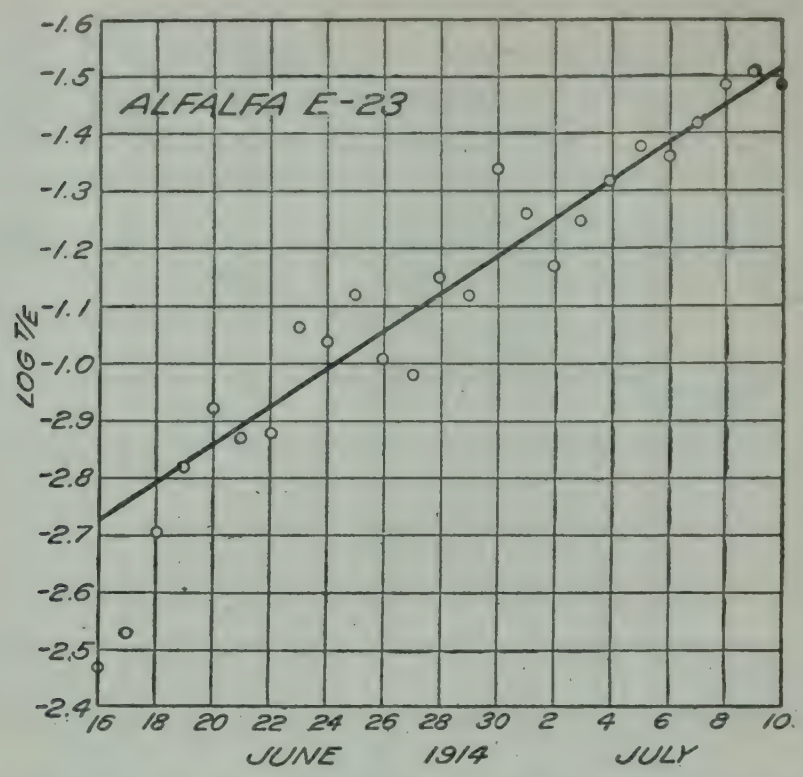


FIG. 15.—Graph showing a linear relation between the logarithm of the transpiration-evaporation ratio of Alfalfa E–23 (in the open) and the time.

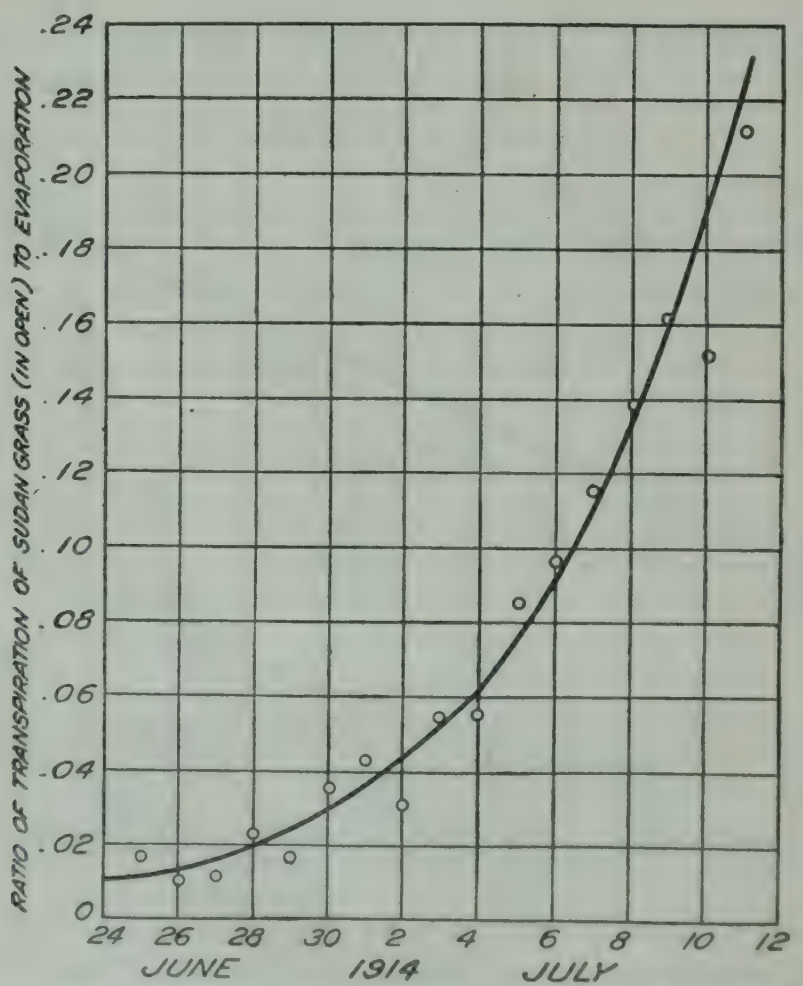


FIG. 16.—Observed daily ratios of transpiration to evaporation during early stages of growth of Sudan grass (shown by circles) compared with exponential graph computed from the relationship shown in figure 11 (solid line).

plotting the ratio of the daily transpiration of one crop to that of the other.

If such ratios depart from unity to any great extent, the difference between the departure of the ratio and that of its reciprocal is so great that the graphs resulting from the plotting of such ratios are not readily com-

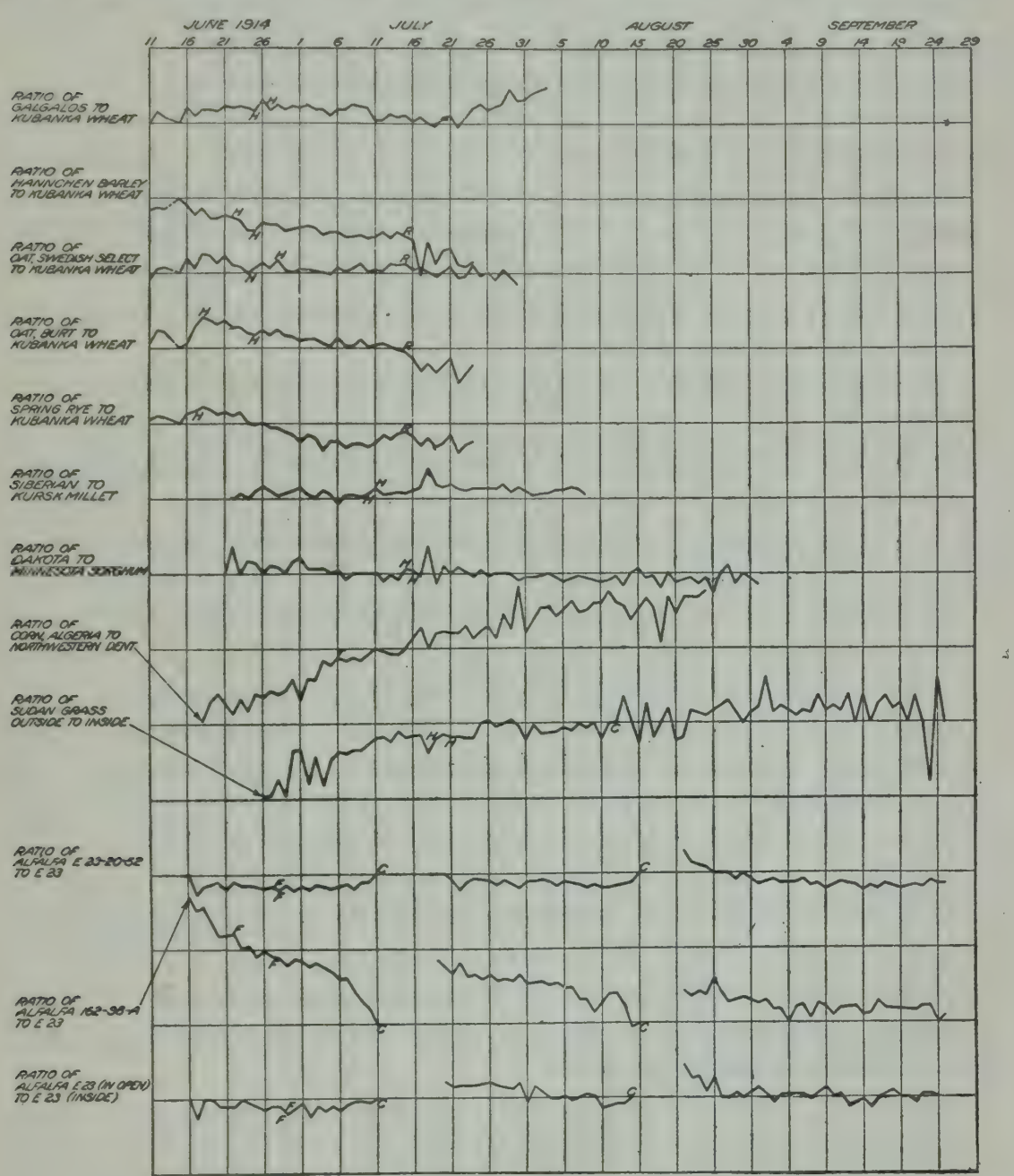

JUNE 1914
JULY
AUGUST
SEPTEMBER
RATIO OF GALGALOS TO KUBANKA WHEAT
RATIO OF HANNCHEN BARLEY TO KUBANKA WHEAT
RATIO OF OAT, SWEDISH SELECT TO KUBANKA WHEAT
RATIO OF OAT, BURT TO KUBANKA WHEAT
RATIO OF SPRING RYE TO KUBANKA WHEAT
RATIO OF SIBERIAN TO KURSK MILLET
RATIO OF DAKOTA TO MINNESOTA SORGHUM
RATIO OF CORN, ALGERIA TO NORTHWESTERN DENT
RATIO OF SUDAN GRASS OUTSIDE TO INSIDE
RATIO OF ALFALFA E 23-20-C2 TO E 23
RATIO OF ALFALFA 152-36-A TO E 23
RATIO OF ALFALFA E 23 (IN OPEN) TO E 23 (INSIDE)

FIG. 17.—Graphs of the daily ratios of the transpiration of the different crops grown in 1914 plotted logarithmically. *F* signifies flowering; *C* indicates the cutting of the crop, which was followed by a new growth from the established root system; *H*, heading; and *R*, ripening.

parable. By plotting the logarithm of the ratio, however, the same departure is obtained for a ratio and its reciprocal. The transpiration ratios of a number of different crops grown in 1914 are shown in figure 17, plotted logarithmically.

The first graph represents the ratio of the daily transpiration of Galgalos wheat to the daily transpiration of Kubanka wheat. The early

ratios are irregular, owing to rainy weather and to the small size of the plants. After this period no difference in the transpiration of the two crops was observable until after July 10, at which time the plants began to show signs of ripening through the dying back of the lower leaves. There was then a sudden drop in the relative transpiration, followed after about two weeks by a gradual increase to harvest time; in other words, Galgalos lost proportionately more water than Kubanka in the final stages of ripening but less in the early stages of ripening.

The ratio of the transpiration of Hannchen barley to Kubanka wheat decreased gradually throughout the whole period. The same was true of Burt oat and spring rye. Swedish Select oat and Kubanka wheat showed only slight differences in the relative transpiration rate. Barley, rye, and Burt oat made their greatest demand on the soil moisture early in the growth season, while the other crops used relatively more water near the latter part of the season.

The graph representing the ratio of Siberian to Kursk millet shows no difference in the behavior of these crops. Dakota and Minnesota Amber sorghum also show no differences except in the early stages of growth. A marked difference is shown in the two varieties of corn included in the 1914 measurements. The ratio increased gradually throughout the season, owing to the fact that Algeria is a late corn compared with Northwestern Dent. The first crop of Sudan grass grown in the open gradually increased its transpiration coefficient with respect to Sudan in the inclosure, but no difference was evident in the second crop.

Alfalfa E23–20–52 and alfalfa E23 showed no differences in their transpiration response. Cuttings were used in alfalfa 162–98A. The plants started more rapidly than the seedlings of alfalfa E23. Not until the third cutting was this advantage fully overcome by the crop grown from seed; in other words, during the period covered by the first two cuttings the transpiration coefficient of alfalfa E23 was gradually increasing compared with that of alfalfa 162–98A. The ratio graphs of the same variety inside and outside the inclosure indicate that the plants inside grew somewhat more rapidly, the graphs of the second and third crops having a downward trend.

The measurements in 1915 included the whole life period of the plants considered (fig. 18). At the beginning and end of the period the amount of transpiration was very small, which results in irregularities in the ratios. The graph representing the ratio of Galgalos wheat to Kubanka wheat corresponds closely with that of 1914, although Kubanka transpired more rapidly than Galgalos in the ripening stages. In 1914 the graph indicates a relatively rapid loss from Galgalos at the end of the ripening season. Galgalos was rusted badly in 1915, and this may account for the comparatively rapid decline in transpiration rate just before harvest.

Two sets of Kubanka wheat were included in the 1915 measurements, which showed the same water requirement (405 ± 6 and 406 ± 3). The second set (pots 109 to 114) produced a heavier crop and was three days later in ripening, which accounts for the rise in the graph at the end of

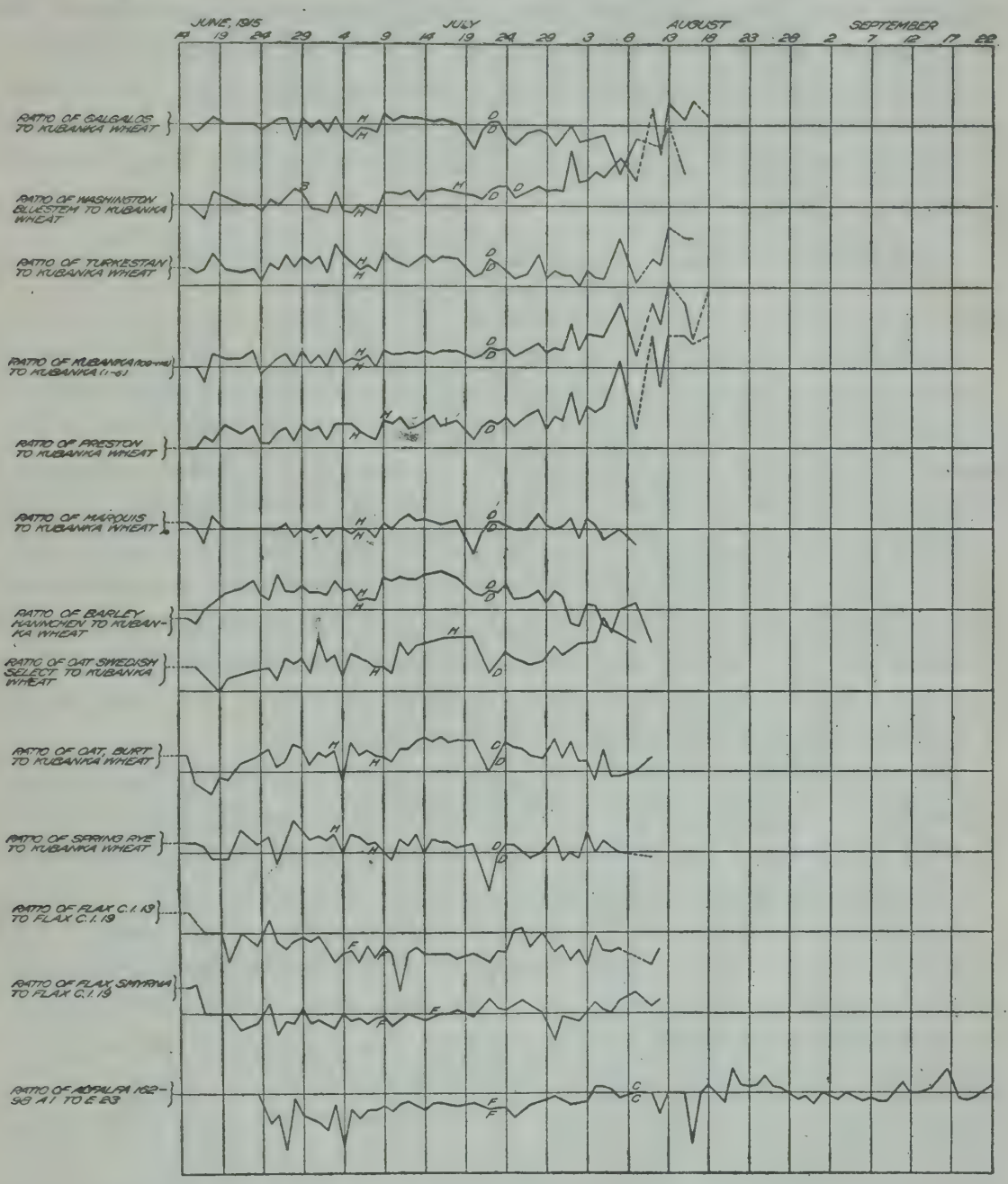

JUNE, 1915
JULY
AUGUST
SEPTEMBER
RATIO OF GALGALOS TO KUBANKA WHEAT
RATIO OF WASHINGTON BLUESTEM TO KUBANKA WHEAT
RATIO OF TURKESTAN TO KUBANKA WHEAT
RATIO OF KUBANKA TO KUBANKA
RATIO OF PRESTON TO KUBANKA WHEAT
RATIO OF MARQUIS TO KUBANKA WHEAT
RATIO OF BARLEY HANNCHEN TO KUBANKA WHEAT
RATIO OF OAT SWEDISH SELECT TO KUBANKA WHEAT
RATIO OF OAT, BURT TO KUBANKA WHEAT
RATIO OF SPRING RYE TO KUBANKA WHEAT
RATIO OF FLAX C.I. 19 TO FLAX C.I. 19
RATIO OF FLAX SMYRNA TO FLAX C.I. 19
RATIO OF ALFALFA TO E.B.3

FIG. 18.—Graphs of the daily ratios of the transpiration of the different crops grown in 1915 plotted logarithmically. *H* signifies heading; *D*, lower leaves dying; *S*, that the plants were forming shoots; *F*, flowering; and *C*, the cutting of the crop, which was followed by a new growth from the established root system.

the season. The rise of the ratio graph is even more marked in Washington Bluestem and Preston, owing to the fact that they ripened five days later than Kubanka. Turkestan showed a relatively greater transpiration than Kubanka early in the season, while Marquis is nearly identical with Kubanka throughout.

Barley and Burt oat increased in transpiration more rapidly and ripened more rapidly than wheat. The ratio graph for Swedish Select oat shows a steady increase from seedtime to heading, when there is a marked drop, followed at first by a gradual and finally by a rapid rise as the wheat ripened. The graph for rye shows a relatively greater transpiration than Kubanka wheat early in the season, decreasing as the season advances.

No marked difference is shown in the flax varieties. C. I. 13 ripened nine days ahead of the other varieties. Except for a gradual increase in the ratio graph throughout the first crop, alfalfa E23 and 162–98A1 showed no marked differences.

CORRELATION OF DAILY TRANSPIRATION WITH WEATHER FACTORS AND WITH EVAPORATION

The correlation of the daily transpiration of a plant with the intensity of the weather factors presents difficulties, owing to the fact that the transpiration coefficient undergoes a gradual change from seed time to harvest. (See march of transpiration, p. 189.) It is consequently necessary in a correlation study to eliminate as far as possible effects due to changes in the size of the plant and to ripening processes. This can be accomplished by comparing the ratio of the transpiration on consecutive days with the ratio of the intensity of a given weather factor for the corresponding days. Since the fluctuations in transpiration from day to day are large in comparison with the daily change in the transpiration coefficient, the effect of the latter is thus minimized.

The use of direct ratios is not, however, wholly free from objection, owing to the fact that the departure of the ratio from unity is not the same in both directions. Transpiration ratios less than unity will be confined in the transpiration table to classes lying between 0 and 1, while transpiration ratios greater than unity have infinity as their upper limit. This can be avoided by correlating the logarithms of the ratios, instead of the ratios themselves, the departure of the logarithm being independent of the direction in which the ratio is taken. As an example, consider three successive days during which the transpiration is 2, 8, and 2 kgm., respectively. The ratio of the first to the second is 0.25, while that of the second to the third is 4. The departure of the ratio from unity is 0.75 in the first case and 3 in the second, while the logarithms of the ratios, -1.3979 (that is, -0.6021) and 0.6021, respectively, show the same departure from zero.

Since it was not practicable to determine the daily transpiration with an accuracy greater than 0.1 kgm., the uncertainty of the ratio of the transpiration on consecutive days increases as the daily transpiration decreases. For this reason only pairs of terms in which at least one of the pair showed a transpiration of 0.6 kgm. or more have been used in the correlation tables.

In all cases where the available transpiration measurements were sufficiently numerous to justify the procedure, the correlation has been

determined between the daily transpiration of each crop and the radiation, temperature, wet-bulb depression, wind velocity, and evaporation from the shallow and from the deep tanks, making 6 correlation tables for a crop, or about 200 tables in all. In order to avoid in the correlation studies the error arising from the change in the transpiration coefficient as the crops develop, the writers have, as outlined above, compared the transpiration ratio on consecutive days with the ratio of the intensity of the weather factor on corresponding days. The difficulty arising from the asymmetry of these ratios has been avoided by taking the logarithm of the ratio in each case. This is, of course, equivalent to taking the difference of the logarithms of the two terms of the ratio, which was the procedure actually followed. In other words, the data in Tables I and III were first converted to a logarithmic basis, and the logarithmic differences then determined day by day. In brief, then, the actual correlation is between the logarithm of the transpiration ratio on consecutive days and the logarithm of the ratio of the intensity of the given weather factor on corresponding days. The transpiration of a crop on a given day thus normally enters twice into the correlation table for that crop, once as the numerator and once as the denominator of the ratios involving the transpiration on consecutive days. The results of the correlation studies are summarized in Tables XXI to XXIII, inclusive.

Small Grains.—The small grains in Table XXI show such similarity in the response of transpiration to various weather factors that the group may profitably be considered as a whole. The correlation coefficients are higher in 1914 than in 1915. It will be recalled that the latter season was cooler, more cloudy, and included many more rainy days. The correlation of transpiration with air temperature is usually, in 9 cases out of 12, slightly higher than with radiation, but the difference is usually less than its probable error.

The correlation coefficients of transpiration of the small grains with wet-bulb depression and with evaporation (shallow tank) show a striking agreement. Considered as a group, they are markedly higher than the correlations with either radiation or temperature, and constitute the highest correlations in the series. Their agreement appears to be due, in part at least, to the fact that the depression in the wet-bulb temperature is dependent upon the rate of evaporation from the muslin covering. It is of interest in this connection to recall the difference in exposure of the wet-bulb instrument and the shallow tank, the former being shaded from solar radiation, while the tank was blackened and fully exposed to the sun's rays.

The evaporation from the deep tank showed a lower correlation with transpiration than the shallow tank. The deep-tank evaporation is correlated with transpiration approximately to the same extent as radiation or temperature. In 1915 the correlation of the deep-tank evaporation with transpiration was markedly lower for many of the small grains.

TABLE XXI.—*Correlation of transpiration of small grains with intensity of weather factors and with evaporation*

Plant.	Year.	Radiation.	Temperature.	Wet-bulb depression.	Evaporation.		Wind.
					Shallow tank.	Deep tank.	
Wheat, Kubanka	1914	0.64±0.06	0.72±0.05	0.88±0.02	0.85±0.03	0.76±0.04	0.26±0.09
Do	1915	.57± .08	.66± .06	.77± .05	.73± .06	.55± .07	.26± .09
Wheat, Galgalos	1914	.63± .06	.73± .05	.86± .03	.86± .03	.70± .05	.29± .09
Do	1915	.55± .08	.64± .06	.85± .04	.81± .04	.67± .06	.25± .09
Oat, Swedish Select	1914	.66± .06	.69± .05	.86± .03	.85± .03	.78± .04	.17± .10
Do	1915	.62± .07	.55± .06	.67± .06	.74± .05	.47± .07	.21± .09
Oat, Burt	1914	.69± .06	.74± .05	.89± .02	.90± .02	.75± .05	.21± .10
Do	1915	.66± .07	.64± .06	.80± .05	.89± .03	.64± .06	.15± .10
Barley, Hannchen	1914	.61± .07	.66± .06	.85± .03	.86± .03	.72± .05	.19± .11
Do	1915	.49± .09	.63± .06	.71± .06	.71± .06	.49± .07	.13± .10
Rye, spring	1914	.65± .06	.73± .05	.94± .01	.91± .02	.77± .04	.19± .10
Do	1915	.65± .06	.55± .06	.73± .05	.79± .04	.47± .07	.15± .09
Mean of 1914 coefficients	.....	.65	.71	.88	.87	.75	.22
Mean of all coefficients	.....	.62	.66	.82	.82	.65	.21
Square of mean of 1914 coefficients	.....	.42	.50	.77	.76	.56	.05
Square of mean of all coefficients	.....	.38	.44	.67	.67	.42	.04

Wind velocity showed during both years a very low correlation with transpiration.

The relative influence of the various climatic factors on transpiration, as expressed by these correlations, may now be considered. If these factors are regarded as independent causative elements in transpiration, the dependence is shown by the square of the correlation coefficient. On this basis the transpiration of the small grains during the two seasons was determined by the different weather factors as follows: Radiation, 38 per cent; temperature, 44 per cent; wet-bulb depression, 67 per cent; wind, 4 per cent. The fact that the sum of these coefficients exceeds unity is the result of intercorrelation among the weather factors.

The association of the transpiration of the small grains with evaporation (shallow tank) is 67 per cent; or the same as with wet-bulb depression. The evaporation from the deep tank shows an association of 42 per cent with transpiration.

MILLET, CORN, AND SORGHUM.—The correlation of the transpiration of millet, corn, and sorghum with the several climatic factors is given in Table XXII. As in the case of the cereals, the coefficients are higher for 1914 than for 1915. The correlation coefficients of the transpiration of these crops with the intensity of the various weather factors show the same relationships disclosed in the case of the cereals. The highest correlation is obtained with wet-bulb depression and with the evaporation from the shallow tank. A somewhat lower correlation is obtained in the case of temperature, radiation, and evaporation (deep tank), which are correlated with transpiration nearly equally. Wind shows as before a very low correlation with transpiration.

TABLE XXII.—*Correlation of transpiration of millet, corn, sorghum, and Sudan grass with intensity of weather factors and with evaporation*

Plant.	Year.	Radiation.	Temperature.	Wet-bulb depression.	Evaporation.		Wind.
					Shallow tank.	Deep tank.	
Millet, Kursk	1914	0.54±0.07	0.61±0.06	0.77±0.04	0.68±0.05	0.66±0.05	0.23±0.09
Do	1915	.58± .07	.63± .06	.70± .05	.78± .04	.49± .08	.19± .10
Millet, Siberian	1914	.57± .06	.64± .06	.78± .04	.73± .04	.69± .05	.31± .08
Sorghum, Minnesota Amber	1914	.61± .05	.64± .05	.79± .03	.70± .04	.59± .05	.16± .08
Do	1915	.64± .08	.38± .12	.57± .10	.75± .06	.48± .11	.07± .14
Sorghum, Dakota Amber	1914	.56± .06	.72± .04	.81± .03	.74± .04	.52± .06	.17± .08
Sudan grass (in inclosure)	1914	.55± .06	.84± .03	.83± .03	.93± .01	.75± .04	.52± .07
Do	1915	.64± .08	.28± .12	.64± .08	.75± .06	.37± .11	.04± .13
Sudan grass (in open)	1914	.52± .07	.81± .03	.85± .03	.82± .03	.60± .06	.32± .08
Corn, Northwestern Dent	1914	.80± .04	.71± .04	.81± .03	.79± .03	.69± .05	.28± .08
Corn, Algeria	1914	.62± .06	.79± .04	.88± .02	.85± .03	.75± .04	.33± .09
Mean of 1914 coefficients	.....	.60	.72	.82	.78	.66	.29
Mean of all coefficients	.....	60	.64	.77	.77	.60	.23
Square of mean of 1914 coefficients	.....	.36	.52	.67	.61	.44	.08
Square of mean of all coefficients	.....	.36	.41	.59	.59	.36	.05

The dependence of transpiration on the various factors, as expressed by the squares of the mean correlation coefficients, are as follows: Radiation, 36 per cent; temperature, 41 per cent; wet-bulb depression, 59 per cent; and wind, 5 per cent. The association of transpiration with evaporation from the shallow tank is 59 per cent, and from the deep tank 36 per cent. As in the case of the cereals, the evaporation from the shallow tank and the integrated wet-bulb depression show the same degree of association with the transpiration.

LEGUMES.—The correlations of the transpiration of the legumes (Table XXIII) with the various climatic factors and with evaporation discloses the same relationships appearing in the other groups.[1] Wet-bulb depression and evaporation from the shallow tank gave, as before, the highest coefficients. Radiation, temperature, and evaporation (deep tank) show a somewhat lower correlation, while wind again shows a low correlation.

The correlation of the transpiration with the intensity of the physical factors of the legumes is lower than either group considered above, but the different factors stand in about the same relationship. The dependence of transpiration on the several climatic factors and the association

[1] The correlation coefficients between the transpiration of amaranthus and the weather factors were as follows:

	1914	1915
With radiation	0.40±0.09	0.69±0.07
With temperature	.45± .08	.60± .09
With wet-bulb depression	.60± .07	.80± .05
With evaporation (shallow tank)	.56± .06	.58± .09
With evaporation (deep tank)	.47± .08	.62± .08
With wind velocity	.04± .10	.15± .14

of transpiration with evaporation as shown by the square of the mean correlation coefficients is as follows: Evaporation (shallow tank), 55 per cent; wet-bulb depression, 50 per cent; evaporation (deep tank), 35 per cent; temperature, 32 per cent; radiation, 27 per cent; and wind, 6 per cent.

TABLE XXIII.—*Correlation of transpiration of legumes with intensity of weather factors and with evaporation*

Plant.	Year.	Radiation.	Temperature.	Wet-bulb depression.	Evaporation.		Wind.
					Shallow tank.	Deep tank.	
Cowpea	1914	0.56±0.06	0.72±0.04	0.82±0.03	0.79±0.03	0.73±0.04	0.31±0.08
Do	1915	.75± .05	.38± .09	.69± .06	.82± .04	.57± .07	.04± .11
Lupine	1914	.58± .06	.63± .06	.75± .04	.76± .04	.68± .05	.36± .08
Alfalfa, E23–20–52	1914	.40± .06	.48± .06	.67± .04	.67± .04	.54± .05	.28± .07
Alfalfa, 162–98A	1914	.42± .06	.45± .06	.67± .04	.69± .04	.55± .05	.25± .07
Do	1915	.52± .07	.74± .04	.65± .05	.78± .04	.61± .06	.19± .09
Alfalfa, E23 (in inclosure)	1914	.43± .06	.50± .06	.70± .04	.74± .03	.56± .05	.32± .07
Do	1915	.52± .06	.71± .04	.66± .05	.69± .06	.50± .06	.18± .08
Alfalfa, E23 (in open)	1914	.47± .06	.55± .05	.76± .03	.72± .04	.58± .05	.31± .07
Mean of 1914 coefficients		.48	.56	.73	.73	.61	.31
Mean of all coefficients		.52	.57	.71	.74	.59	.25
Square of mean of 1914 coefficients		.23	.31	.53	.53	.37	.10
Square of mean of all coefficients		.27	.32	.50	.55	.35	.06

CORRELATION OF DAILY TRANSPIRATION OF ALL CROPS, CONSIDERED AS A SINGLE POPULATION, WITH THE INTENSITY OF WEATHER FACTORS

The degree of correlation of the various weather factors with the transpiration ratios of all the plants considered as one population has also been determined. The coefficients for the two years are given in Table XXIV, together with their squares. They show the same relationships as in the crop groups. The mean values of the squares of the correlation coefficients for the two years are as follows: Wet-bulb depression, 0.55; temperature, 0.38; radiation, 0.30; wind, 0.04; evaporation (shallow tank), 0.56; and evaporation (deep tank), 0.33.

TABLE XXIV.—*Correlation of transpiration of all crops, considered as a single population, with weather factors, based on logarithmic differences of consecutive days*

Weather factor.	1914		1915	
	Correlation coefficient.	Square of correlation coefficient.	Correlation coefficient.	Square of correlation coefficient.
1. Radiation	0.50±0.01	0.25	0.59±0.02	0.35
2. Temperature	.64± .01	.41	.59± .01	.35
3. Wet-bulb depression	.79± .01	.62	.69± .01	.48
4. Evaporation (shallow tank)	.72± .01	.52	.75± .01	.59
5. Evaporation (deep tank)	.63± .01	.40	.51± .02	.26
6. Wind velocity	.26± .02	.07	.14± .02	.02

A SECOND METHOD OF CORRECTING FOR THE CHANGE IN THE TRANSPIRATION COEFFICIENT IN DETERMINING THE CORRELATION OF TRANSPIRATION WITH WEATHER FACTORS

The effect of the change in the transpiration coefficient can also be largely avoided in correlation studies involving transpiration through recourse to the transpiration-evaporation ratio. If transpiration and evaporation show the same hourly response to weather factors, the transpiration-evaporation graph for the season would represent the progressive changes in the transpiration coefficient. But since the transpiration-evaporation graph always presents irregularities owing to experimental errors and to the fact that a one-to-one correspondence does not exist between the two quantities, it is necessary to make use of a smoothed curve through the observations in applying this reduction. The ratios for each day of the ordinate of the smoothed graph to the maximum ordinate will give the transpiration coefficient for each day in terms of the maximum.[1] If, now, we divide the transpiration observed on each day by the corresponding ratio of the ordinates, we obtain a series of daily transpiration quantities which are independent of the size and degree of maturity of the plant, but which still retain all the daily fluctuations due to environment. These corrected transpiration quantities can, therefore, be used directly in studying the correlation of transpiration with any given environmental factor.

In the case of Kubanka wheat, the smoothed transpiration-evaporation ratio was represented by two straight lines meeting in a maximum on July 12. The daily transpiration of Kubanka has been corrected on this basis for the march in the transpiration coefficient and the correlation with the various physical factors determined. The results of the computations are given in Table XXV. For comparison, the correlation coefficients based on the first method (ratios of values on consecutive days) have also been included in Table XXV. It will be seen that the coefficients determined by the first method are slightly higher than those based on the second method. This is to be expected, since the second method assumes a one-to-one correspondence between transpiration and evaporation, so that departures from such a relationship distort the computed transpiration. The difference in the coefficients computed by the two methods is, however, usually less than its probable

[1] The daily ratios of the observed transpiration and evaporation can not be used directly, as the following discussion will show:

Let $\frac{T_t}{E_t}$ represent the ratio of the transpiration to evaporation at time t, and $\frac{T\max}{E\max}=k$, represent the maximum ratio.

The daily ratio at time t expressed in terms of the maximum is then $\frac{T_t}{kE_t}$.

If, now, we divide the observed transpiration T_t by this ratio in order to free the daily observations from the effect of the change in the transpiration coefficient, we obtain as the quotient simply kE_t. In other words, the observed transpiration is dropped from consideration, and the specific assumption is made that transpiration is proportional to evaporation. The use of the smoothed graph avoids this assumption so far as the transpiration of any given day is concerned.

error, and the correlations are substantially in accord with those previously determined.

TABLE XXV.—*Comparison of correlations obtained by the two methods of correcting for the march in the transpiration coefficient, Kubanka wheat, 1914*

Factor	First method	Second method
Transpiration and evaporation:		
Shallow tank	0.85±0.03	0.83±0.03
Deep tank	.76± .04	.67± .06
Transpiration and radiation	.64± .06	.77± .03
Transpiration and temperature	.72± .05	.62± .07
Transpiration and wet-bulb depression	.88± .02	.79± .04
Transpiration and wind velocity	.26± .09	.27± .10

SUMMARY

The transpiration studies included in this paper were made at Akron, Colo., during the summers of 1914 and 1915.

The plants were grown in large pots (115 kgm. of soil) and sealed to prevent evaporation from the soil surface.

The pots were weighed each morning before the transpiration response to sunlight had set in.

Six pots of each crop were used in the determinations, and were weighed to 0.1 kgm.

Twenty-two crops (132 pots) were included in the 1914 measurement and 23 crops (138 pots) in 1915.

Continuous automatic records were also obtained of air temperature, solar radiation, wet-bulb depression, wind velocity, evaporation from a shallow tank, and evaporation from a deep tank.

The climatic conditions were exceptionally uniform throughout the season of 1914. The summer of 1915 was unusually rainy.

During a 10-day period of maximum transpiration the annual crops lost about one-fourth of the total water lost during the season. The alfalfas lost during this period almost one-half of the total water transpired in the production of the second crop.

During a 10-day period of maximum transpiration the daily loss of water from the small grains ranged from 12 to 16 times the dry weight of the crop; millets, corn, and sorghums, 6 to 9 times; and alfalfas, 36 to 56 times the dry weight harvested. On the basis of a production of 1 ton of dry matter per acre, this would correspond in the case of the small grains to a daily loss of 0.11 to 0.14 acre-inch of water; corn, millet, and sorghum, 0.05 to 0.08 acre-inch; and alfalfas, 0.32 to 0.49 acre-inch.

The loss of water from the small grains during the period of maximum transpiration amounted to 1.5 kgm. per square meter of plant surface per day; Sudan grass, 0.8 kgm; and alfalfa, 1.6 kgm. This is from 5 to 14 per cent of the loss during the same period from a free water surface of equal area.

The transpiration of the different crop plants per unit area of plant surface shows less variation than the transpiration per unit weight of dry matter. In other words, the greater efficiency shown by certain plants in the use of water appears to be due more to a reduction in plant surface than to a reduction in transpiration per unit area of surface. The direct solar radiation received by the plants at Akron is usually not sufficient to account for the observed transpiration during the midday hours. In some of the small grains the energy dissipated through transpiration is twice the amount received directly from the sun.

The march of the transpiration due to changes in the plant alone (change in the transpiration coefficient) may be expressed by the ratio of the daily transpiration to the daily evaporation if we assume the latter to constitute a perfect summation of the weather conditions determining transpiration. The transpiration of the annual crop plants (aside from fluctuations due to weather) rises to a maximum a little beyond the middle of the growth period and then decreases until the plants are harvested. Perennial forage crops such as alfalfa increase steadily in transpiration to a maximum at or near the time of cutting. Various crops show their individuality by departing more or less from these types.

The transpiration coefficient of many of the crops increases exponentially during the early stages of growth. Sudan grass, for example, doubled its transpiration coefficient every four days during the early growth period. Alfalfa throughout practically the whole period between cuttings doubled its transpiration every eight days.

The relative change in the transpiration coefficients of two crops may be determined by taking the ratio of the transpiration of the two crops day by day without the necessity of correcting for changes in weather.

The correlation has been determined between the various physical factors of environment and the transpiration of the different crops, considered both individually and as one population. The correlation coefficients in the latter case for the season of 1914 are as follows:

Transpiration with radiation, 0.50 ± 0.01; with temperature, 0.64 ± 0.01; with wet-bulb depression, 0.79 ± 0.01; with evaporation (shallow tank), 0.72 ± 0.01; with evaporation (deep tank), 0.63 ± 0.01; and with wind velocity, 0.26 ± 0.01.

The small grains show individually a markedly higher correlation between transpiration and the intensity of the various physical factors than was observed when all the crops were combined in one population. The mean correlation coefficients for the small grains (1914) are as follows: Transpiration with radiation, 0.65; with temperature, 0.71; with wet-bulb depression, 0.88; with evaporation (shallow tank), 0.87; with evaporation (deep tank), 0.75; with wind velocity, 0.22.

The corn, sorghum, and millet group and the legume group show a somewhat lower correlation between transpiration and the intensity of the physical factors of environment. The plants in the various groups,

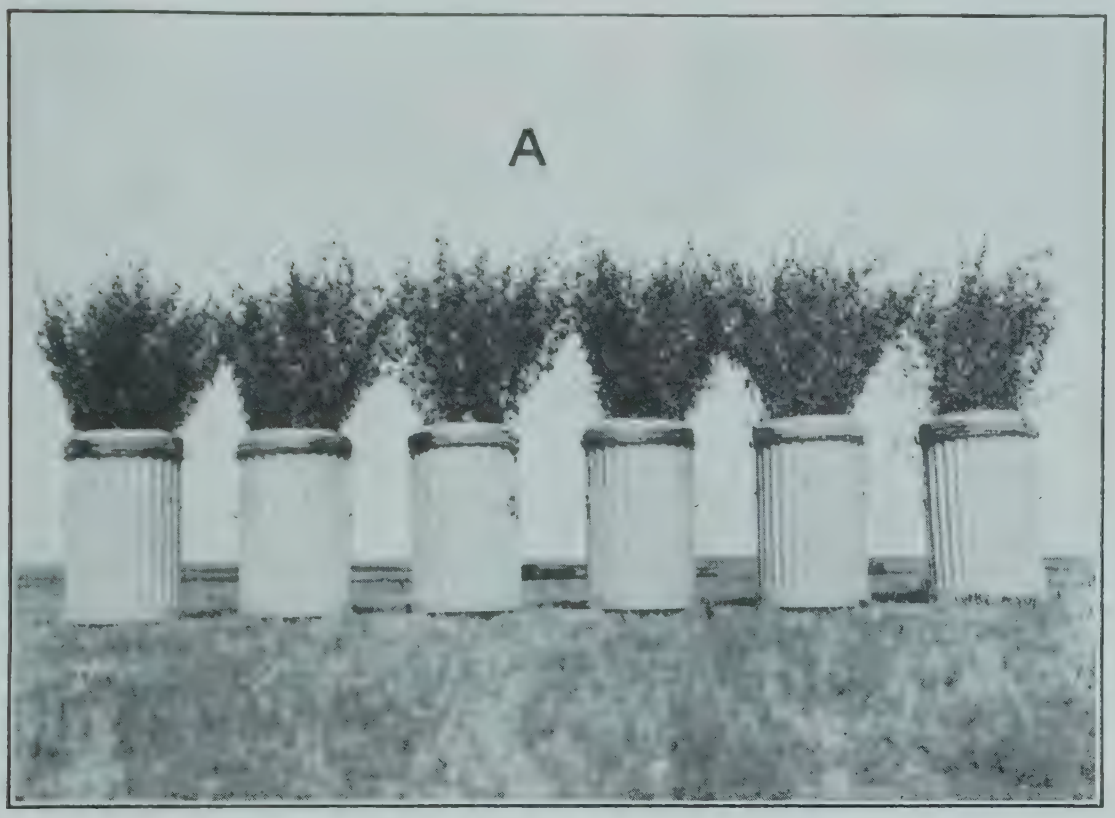
however, show the same relative dependence of transpiration upon the physical factors. Wet-bulb depression and evaporation (shallow tank) exhibit the highest correlation with transpiration in all cases, while wind velocity is correlated with transpiration to a very slight extent at Akron.

The degree of dependence of transpiration of the small grains in 1914 upon radiation, temperature, wet-bulb depression and wind velocity, considered as independent causative factors, as shown by the squares of the correlation coefficients is as follows: Wet-bulb depression, 0.77; temperature, 0.50; radiation, 0.42; and wind velocity, 0.05. Since the sum of these squares exceeds unity, the physical factors are evidently intercorrelated. The association of transpiration of the small grains with evaporation (shallow tank) is 0.76, or the same as with wet-bulb depression.

PLATE 5

Fig. A.—Six pots of alfalfa used in transpiration measurements. The daily transpiration of each pot of plants was determined independently, and the mean of the six determinations used as the daily transpiration of the crop.

Fig. B.—Six pots of corn used in transpiration studies.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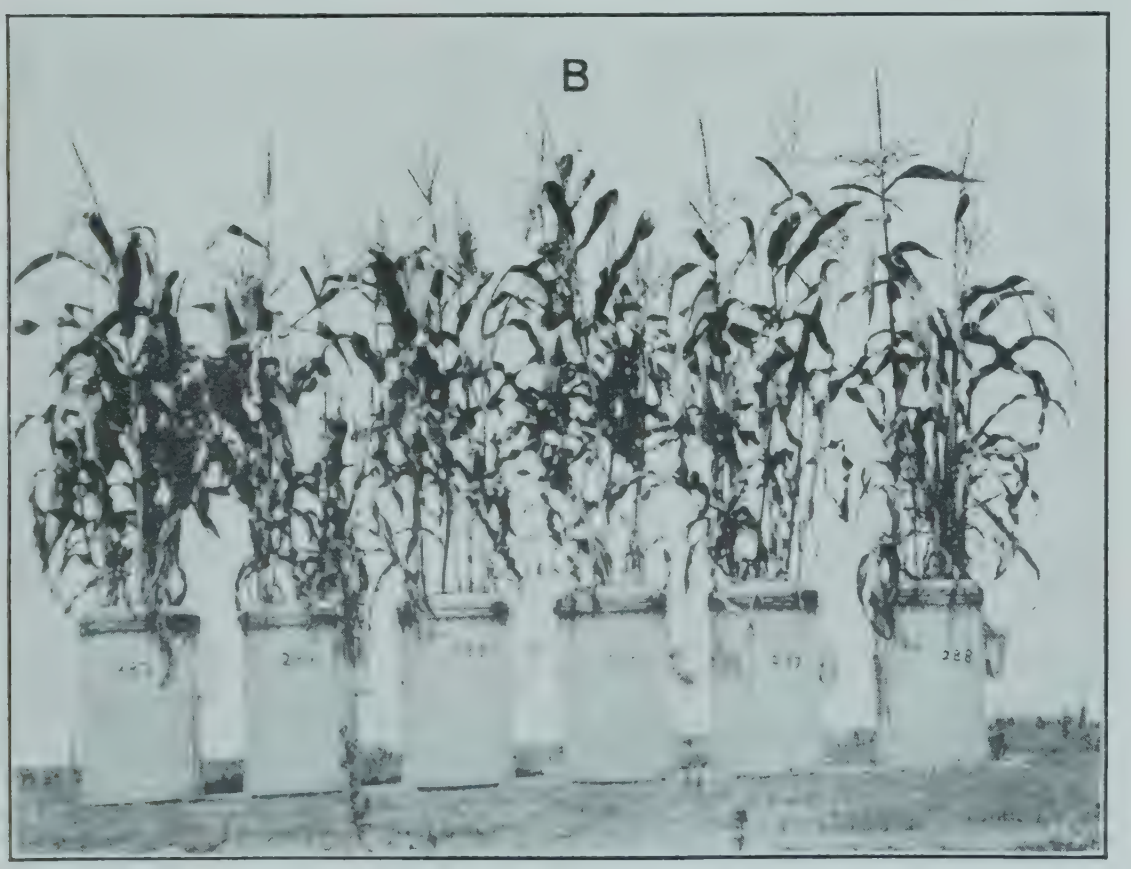
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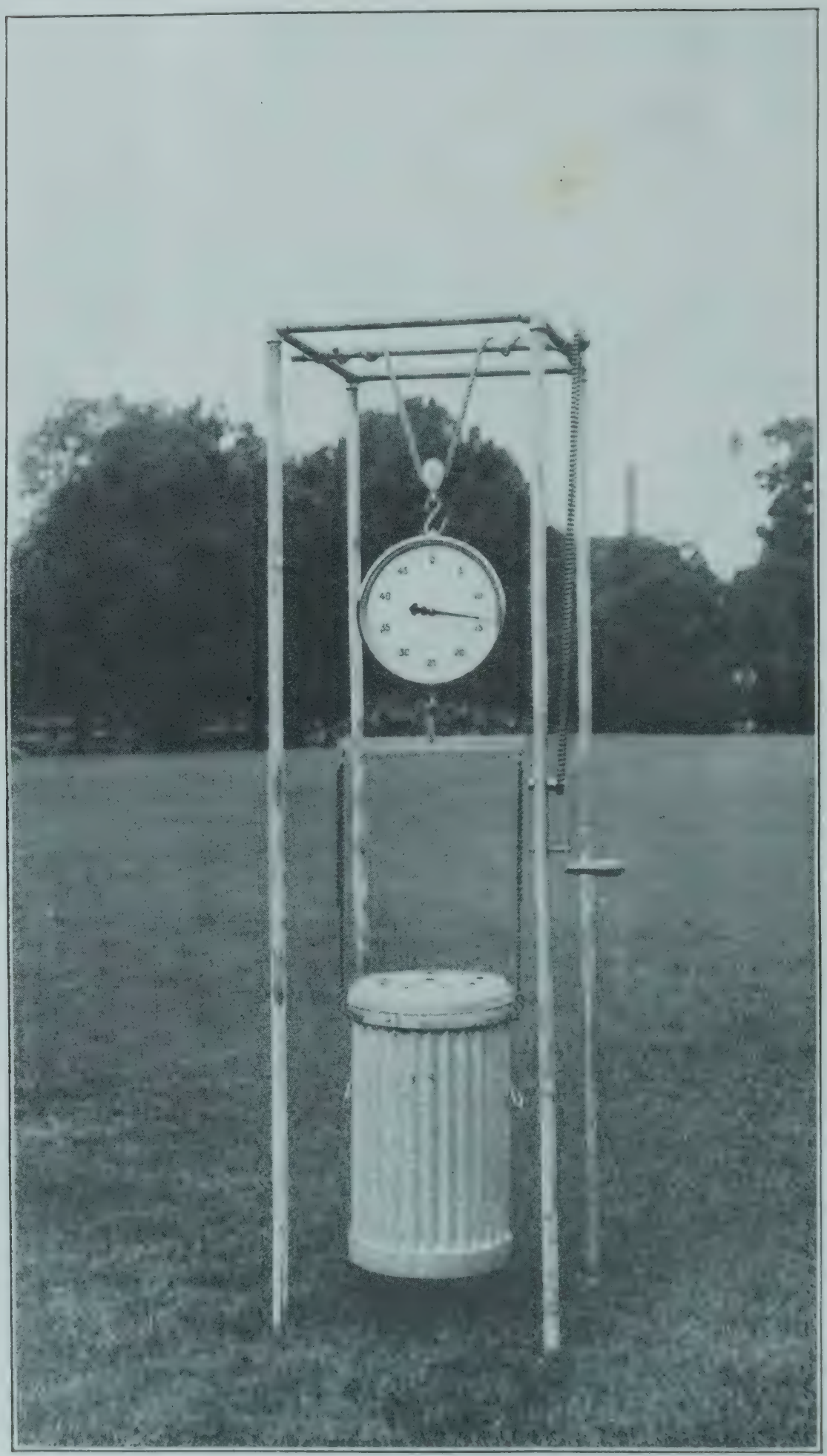

PLATE 6

The type of spring balance and lifting device used in the transpiration measurements. The balance was checked against a standard weight of 130 kgm. each morning before and after the weighings. The pot is 16 inches in diameter and 26 inches high. The cover is provided with 1-inch holes, as used with alfalfa and sorghum. The center hole is used for watering.

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SPONGOSPORA SUBTERRANEA AND PHOMA TUBEROSA ON THE IRISH POTATO

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INTRODUCTION

After finding that *Spongospora subterranea* was well established within our borders, the writers undertook to learn what its effect might be on the American potato (*Solanum tuberosum*) industry, taking up for this purpose the study of such questions as its geographical distribution and the factors governing the same, relation of the fungus to the roots and stems of the plant, and the possibility of its occurrence on other hosts. Such points as its damage to the tubers, relation to soil types, moisture, and control measures have received consideration. Some of these points have not been fully settled, but the data available are published here because of the widespread interest in this disease and its importance in many parts of the United States.

GEOGRAPHICAL DISTRIBUTION OF SPONGOSPORA SUBTERRANEA IN THE UNITED STATES

When *Spongospora subterranea* was discovered in Canada and in Maine, the question as to what would be its distribution in the United States immediately arose. Although quite extensive, European literature regarding the disease caused by this fungus contains but little information as to the factors that determine its geographical distribution. Owing to this fact and to the varied soil and climatic conditions in the United States, the writers were confronted with a new problem, which has been studied by means of surveys, by planting infected seed in many different localities, and by transplanting soil from various Southern States into the infected section of northern Maine.

DISTRIBUTION AS DETERMINED BY SURVEYS

Since the publication in the spring of 1914 of Bulletin 82 of the Department of Agriculture (8)[1], which showed that the disease was then known to

[1] Reference is made by number to "Literature cited," p. 253.

be generally distributed in northern and central Europe and the British Isles and was present in northeastern Canada and northern Maine, the writers, in cooperation with the Federal Horticultural Board and various State agencies, have extended the survey and as a result have found that the disease exists in isolated sections from the Atlantic to the Pacific. Table I gives its distribution in the United States.

TABLE I.—*Present distribution of powdery-scab in the United States*

First collection.		Collector.	Infected area.	Crop season.
Date.	Location.			
June 23, 1913	Presque Isle, Me....	I. E. Melhus.....	Aroostook County Washington County. Penobscot County	1912
Oct. 24, 1914	Chateaugay, N. Y..	.....do.....	Franklin County. Clinton County...	1914
Apr. 26, 1915	Nehalem, Oreg.....	F. D. Bailey.....	Clatsop County... Tillamook County	1914
May 1, 1915	Hastings, Fla.....	I. E. Melhus.....	St. Johns County.	1915
July 23, 1915	Snohomish, Wash...	F. D. Heald.....	Snohomish County.	1914
Sept. 18, 1915	Virginia, Minn.....	C. N. Frey..... E. C. Stakman.....	Carlton County... Lake County..... St. Louis County..	1915

As shown by Table I, there are six infested sections in the United States, all northern, except the one in Florida. This distribution is strikingly similar to that of *Phytophthora infestans* and lies wholly within its geographical range, which is confined to the northern part of the United States and to certain sections of the South in which a potato crop is grown during the winter.

DISTRIBUTION AS DETERMINED BY INFECTED SEED IN VARIOUS LOCALITIES

In order to determine whether *Spongospora subterranea* would develop south of infected sections in Maine and New York, infected seed was planted in 12 different States along the Atlantic seaboard in the spring of 1915, trials being made at more than one point in some States. The experiment was carried on in cooperation with the State pathologists and others interested in potato diseases, and the seed was planted in each case in accordance with the general practice in the respective locality. The results obtained are summarized in Table II.

As shown by Table II, no powdery-scab developed in any of these experiments. In each case all of the suspicious-looking specimens were sent to the writers for examination.

TABLE II.—*Results of planting seed infected with Spongospora subterranea outside the infected area*

Locality.	Grower.	Number of hills.	Result.
Arlington, Va.	Bureau of Plant Industry.	2,944	No powdery-scab.
West Raleigh, N. C.	H. R. Fulton.	80	Do.
New Brunswick, N. J.	M. T. Cook.	242	Do.
College Park, Md.	J. B. S. Norton.	100	Do.
Gainesville, Fla.	H. E. Stevens.	100	Do.
Ithaca, N. Y.	M. F. Barrus.	72	Do.
East Marion, N. Y.	F. V. Rand.	48	Do.
Geneva, N. Y.	F. C. Stewart.	50	Do.
Newark, Del.	T. F. Manns.	60	Do.
Amherst, Mass.	A. V. Osmun.	18	Do.
Norfolk, Va.	T. C. Johnson.	200	Do.
Clemson College, S. C.	H. W. Barre.	100	Do.
New Haven, Conn.	G. P. Clinton.	460	Do.
State College, Pa.	C. R. Orton.	200	Do.
Morgantown, W. Va.	N. J. Giddings.	81	Do.

As the season of 1915 was especially wet and still no *Spongospora subterranea* developed in any of the various localities named, it seems safe to assume that its chances for development in seasons with normal rainfall would be still more unfavorable.

Each of the trials referred to was on a small scale and for one season only, except that at Arlington, Va., which was carried through two seasons. In this trial 13 rows were planted in 1914 and 10 in 1915, or in all about 2,944 hills, with infected seed, and also, in 1915, 3 rows, or 384 hills, with clean seed in soil which had been planted with infected seed in 1914. In none of these rows was a single tuber found to be infected with *Spongospora subterranea;* consequently it seemed safe to assume that the conditions were not favorable to the disease, and this raised the question as to what constitutes the limiting conditions.

DISTRIBUTION AS DETERMINED BY TRANSPLANTING SOIL FROM OTHER STATES INTO NORTHERN MAINE

The influence of climate was studied by obtaining 200-pound lots of soil from 12 of the plots that had been planted with infected seed in nine of the States mentioned in Table II, shipping it to northern Maine, and planting it with infected seed of the Irish Cobbler variety. Naturally these soils varied materially in texture and composition, some being extremely light, while others were very heavy. These samples were placed in boxes 2 feet square and 8 inches deep, and the boxes set down into the soil in a field of virgin land where conditions were favorable for the development of *Spongospora subterranea.*

Because of the late arrival of most of the soils, planting was later than is usual in this section—that is, on June 19 in eight cases and on

July 3 in two cases—but otherwise the procedure was practically the same as that generally followed in northern Maine, and the plants received, so far as possible, the same cultural treatment as potatoes growing under field conditions. Owing to the late planting and early frost, the plants had a rather short growing season; and although not harvested until October 15, the tubers were small and in most cases immature. However, the conditions were sufficiently favorable to permit the development of the disease, which was the primary object of the experiment, and the results from this standpoint are given in Table III.

TABLE III.—*Results of planting potatoes infected with Spongospora subterranea at Presque Isle, Me., in soil from the plots in which diseased seed had been grown in various States*

Source of soil.	Number of hills.	Number of sound tubers.	Tubers infected—	
			Number.	Per cent.
Diamond Springs, Va.	1	3	2	40.00
Amherst, Mass.	3	15	3	16.66
College Park, Md.	2	9	0	0
Morgantown, W. Va.	4	12	4	25.00
New Brunswick, N. J.	2	11	3	21.40
Unknown [a]	2	10	1	9.00
Geneva, N. Y.	1	8	0	0
Mount Carmel, Conn.	3	16	2	11.11
Gainesville, Fla.	2	14	1	6.66
Ithaca, N. Y.	2	8	3	27.27
Newark, Del.	2	15	0	0
Unknown [a]	1	7	0	0
Presque Isle, Me.	2	8	2	20.00

[a] In both of these cases the tags showing the source had been lost en route, which made it impossible to say definitely whence they came.

From the fact that *Spongospora subterranea* developed in 8 of the 12 soils tested, as shown by Table III, it seems safe to assume that it would have resulted in all had it been possible to have more soil and to make conditions more favorable for the growth and development of the potato plant. However, the experiment developed the important fact that powdery-scab was produced under Maine conditions in soils in which in their native States the disease was not produced.

This raised the question as to whether soil from infected sections will produce an infected crop when transplanted into a noninfected section. To test this, two lots of soil of 200 pounds each were collected from a field in northern Maine that produced an infected crop in 1914, and one was shipped to Washington, D. C., and was used in growing potatoes in the green house, and the other to Norfolk, Va., where it was placed in large iron cylinders sunk into the ground at the edge of a potato field and planted with clean tubers. The former produced a crop that became badly infected with powdery-scab, while the plants produced in the latter developed unusually well and the progeny re-

mained totally free from powdery-scab. This experiment indicates that when transplanted outside of the infected section infected soil does not yield an infected crop.

CLIMATIC CONDITIONS THE CONTROLLING FACTOR IN DISTRIBUTION

Unless climatic conditions are suitable, *Spongospora subterranea* will not develop, as is shown in the preceding pages. The studies in Maine show that periods of rainfall, followed by cool, damp, cloudy weather during the growing season, are periods of infection and are highly essential to the development of the disease. Such periods prevail during the growing season not only in the northern sections, but also in Florida, where potatoes are planted in January and February and harvested in April and May. Moreover, much of the infected area in Florida is in a section in which irrigation is practiced, the water there being supplied from artesian wells. The water level here is within a few inches of the surface for days at a time. Table IV shows the rainfall in the six infected areas during one growing season.

TABLE IV.—*Average monthly precipitation in powdery-scab-infected areas during the growing season of 1914 or 1915*

State.	Locality.	Period.	Average rainfall.
			Inches.
Florida	Hastings (St. Augustine).	February–July, 1915	4.20
Washington	Snohomish	April–October, 1914	2.50
Oregon	Astoria	do	3.64
Do	Glenora	May–October, 1914	4.70
Minnesota	Duluth	April–September, 1915	2.47
Do	Virginia	do	2.79
New York	Chateaugay (Dannemora).	April–October, 1914	2.80
Maine	Presque Isle	do	3.00
Do	do	April–October, 1915	3.08
Do	Van Buren	April–October, 1914	3.46
Do	do	April–September, 1915	3.38

PREVALENCE AND PERIOD OF EXISTENCE OF SPONGOSPORA SUBTERRANEA IN THIS COUNTRY

In Aroostook County, Me., *Spongospora subterranea* exists on many farms. In most cases the disease caused by this organism occurs in isolated spots, varying from a fraction of an acre to 5 acres; but in other places—for example, sections north and northwest of Caribou, including Perham, New Sweden, and Stockholm—infection is quite general. The disease is always most prevalent on wet, poorly drained land.

During the harvest season of 1914 several fields were examined; and notwithstanding the fact that fairly clean seed which had been treated with the usual strength of formaldehyde had been used, from 50 to 75 per

cent of the crop was found to be infected with *Spongospora subterranea*. In the case of two barrels of tubers selected at random from a 15-acre field near Caribou that had been planted to potatoes in 1914 after they had been picked by laborers and the healthy and infected tubers separated, 68 per cent by weight were found to be infected with the disease. The field in question was no exception, so far as the prevalence of the disease is concerned. Again, in the case of 117 hills which were dug at the same time by hand at random over 2 acres of this field and the infected and healthy tubers separated, 63 per cent of the progeny was infected and only two hills were free from the disease. It is needless to say that the crop thus infected could not be marketed for table use; and as the only way to turn it into money was to sell it for starch purposes, the grower sustained considerable loss, potatoes for starch usually selling for less than 15 cents per bushel.

The question as to how long land infected with *Spongospora subterranea* will remain contaminated is an interesting one. Pethybridge (12, p. 352), of Ireland, holds that it will remain infected for three years. In the case of two fields which, after growing a crop of potatoes, were in oats for one year and in meadow for four years and were planted to potatoes in 1914, over 50 per cent of the tubers were infected with *S. subterranea*, although there was every assurance that the seed used was free from the disease. Numerous other cases indicate that the disease can live in the soil for more than three years, and from facts at hand the writers believe that it can live for at least five years and probably much longer. It is evident, therefore, that when a piece of land once becomes infected, a very long rotation is necessary to rid it of infection and its value for growing potatoes is materially diminished.

The facts that *Spongospora subterranea* can live in the soil for at least five years and that it is prevalent in certain sections of Maine raise the question as to what length of time it has existed in this country. Its distribution in three counties of Maine, extending from the southern coast to the northern boundary, and in areas scattered over thousands of acres in the State naturally required some time; and consequently it must have existed in this country for a considerable period.

Not only is the disease widely distributed in Maine but also in the adjoining Province of New Brunswick, the St. John River Valley being quite generally infected, and in Prince Edward Island, which has long been settled. As the earlier settlers of northern Maine came from parts of New England farther south and up the St. John River Valley and as more than 60 per cent of the population of the State is of New Brunswick origin it is evident that over half the inhabitants of Maine came from a section generally infected with *Spongospora subterranea*. In view of this fact and that the disease exists in this country and in Canada, it seems reasonable to believe that it has existed in the infected sections of Maine for at least 15 years.

SUSCEPTIBILITY OF ROOTS, STOLONS, AND STEMS OF POTATO PLANTS TO SPONGOSPORA SUBTERRANEA

Although *Spongospora subterranea* is known only as a disease of the tuber of the Irish potato, the fact that its causal organism is a slime mold and that many other species of the family flourish on the root systems of their hosts led the writers to suspect that it infects the other underground parts of the potato. Very meager information on this phase of the subject was obtained from a survey of the literature on the disease. Johnson (5) incidentally mentions seeing pustules on stolons and Pethybridge (12, p. 352) mentions evidence of the disease on roots and sprouts, but in no way do these references show the prevalence and actual relationship. In the fall of 1914 a lot of 200 pounds of soil was collected in a field in which a crop of potatoes infected with *S. subterranea* had just been grown. Some of the soil was placed in 12-inch pots in the greenhouse and planted with potatoes known to be free from powdery-scab. When the crop had been harvested, which was before all the vines were dead, and the root systems carefully washed so that they might be examined for any signs of infection, roots of all sizes were found to be very generally infected with white galls (Pl. 7, fig. C, D) strongly resembling the well-known legume nodules. On sectioning these galls (Pl. 8, fig. A) when nearly mature, they were found to contain a large number of immature spore balls of *S. subterranea.* Similar galls were found on the stolons and main stems of the plant (Pl. 7, fig. A, B), and in two of the pot cultures pustules were found on the stems about 1½ inches above the surface of the soil, this latter infection having taken place probably while the soil covered this portion of the stem.

In one case in which the galls on a single plant were counted, 149 were found on the roots, 19 on nine stolons, and 8 on three stems; besides, some were doubtless lost in the process of disentangling the roots. This plant produced nine tubers, four infected, and five free from *Spongospora subterranea.*

The presence of *Spongospora subterranea* on the roots of potato plants growing in pot cultures in the greenhouse naturally raised the question as to whether infection is as prevalent under field conditions. Careful watch was kept for evidence of the disease on the roots of plants growing in the soil-treated plots on infected soil at Caribou during the summer of 1915. The first infection was not found until August 5, although examinations had been made weekly from the time the plants began to come up. At this time the galls, which were on the small rootlets both near the surface and deep down in the ground, were white and no larger than pinheads; but day by day they became more pronounced until finally they were comparable to those found in the greenhouse. No infections were found on the tubers when first noted on the roots,

although over 200, many as large as hen's eggs, from 47 hills were examined.

On August 11, 100 hills were dug from one row, and on examination galls were found on the roots in every case, and in many instances were more numerous than on the roots of the plants grown in the greenhouse. From the progeny of these hundred hills five tubers were found that showed under the epidermis of the tuber early stages of infection with *Spongospora subterranea*, consisting of purplish brown, fimbriate, bacterial-colony-like spots, some no larger than pinheads. Infections on the roots progressed more rapidly than on the tubers, as shown by the foregoing statements, but whether this was due to the former's having been infected earlier or whether the fungus developed faster on the rootlets is not known.

It is not unusual—in fact, it is very common—for the root system of a potato plant to be quite generally infected while the tubers remain totally free. This occurred in the case of the Green Mountain and Irish Cobbler varieties, which are generally grown in the vicinity of Caribou. It was very common in the variety plots to find the root system badly infected and the tubers absolutely clean. This leads to the natural conclusion that the critical test for the presence of the disease in a field or section is the freedom of the root system, and that the roots and not the tubers are the organs of the plant which determine the resistance of potato plants to the disease.

The development of new galls on the roots in the field ceased before the vines died or were killed by frost. There was an outbreak of root infection on August 5, and this lasted about two weeks; but after that no new galls could be found, and those present matured and broke up into a mass of spore balls of *Spongospora subterranea* in exactly the same way as does the content of the sorus on the tuber. Why new infections did not continue to develop is not well understood, as there were plenty of young tender rootlets and the soil conditions were apparently comparable with those which existed earlier. It may well be that there are host relations and environmental influences that check the disease after it has reached a given stage of development or time of the year.

When infected seed was planted in virgin soil, root infection took place in 57 days; where clean seed was planted in infested soil, it took place in 69 days. The first galls found were on rootlets close to the diseased parent tuber, but later others showed infection. Although not as numerous on the plants grown in infected soil, it was not uncommon to find from 30 to 50 galls on a single plant grown in virgin soil. The periods of infection mentioned do not, of course, represent the incubation period of the disease, because of the fact that some time elapsed before the plants threw out roots. A better idea of the time required for infection and development sufficient for galls to become conspicuous can be formed from the following experiment.

Twelve plants with strong root systems and from 8 to 10 inches high were washed and transplanted from clean to infected land in the field on August 5, the day on which root infection was first discovered, and for three or four days they were well watered and shaded. On August 19 three of the plants were taken up and the roots examined, but only one gall was found. However, when the nine others were dug, on September 3, and the roots examined, from 3 to 11 galls each were found on 6 plants, which suggests that from 14 to 34 days must elapse after the plant reaches a certain stage of development before infection with *Spongospora subterranea* takes place.

A similar experiment was begun on September 7, plants of approximately the same age as those used on August 5 being transplanted from healthy to diseased soil. The plants were dug on October 1 and the root systems and tubers carefully examined, but no infection was found.

The small amount of infection in the case of the first experiment and absence of infection in the second is explained by the fact that very few infections took place on any of the plants on the plots after August 15, as previously noted.

With a view to determine whether the plant must be a certain age before infection can take place, 200 seed pieces were planted on July 26 in infected soil in the field. On August 20, when the plants were 3 inches high and had extensive root systems, 100 hills were dug and examined, but no infection was found, although in an adjoining row planted on May 26 the roots were generally infected, infection having occurred between August 1 and 15. On September 24 the remaining hundred hills were dug and examined, but no infection was found, although doubtless the plants had some roots by August 15, which tends to indicate that the host tissue had not reached the susceptible stage.

Very little is known about the factors that favor infection. Moisture, however, is doubtless an important limiting factor. Lime increases the amount of powdery-scab on the roots and tubers. Root galls were especially large and abundant on the plots that received lime at the rate of 3,000 pounds per acre. That injuries also increase the tendency to infection was indicated by the finding of numerous galls on lesions caused by fungi other than *Spongospora subterranea*. In one case four galls were found on an injured portion of a plant that had been partially broken off and had recovered, the indications being that infection had taken place after the injury.

NEW HOSTS OF SPONGOSPORA SUBTERRANEA

As soon as it was found that *Spongospora subterranea* infects the root system of the potato plant, investigations were undertaken to determine whether it infects the roots of other solanaceous plants. Fifty-three species of solanaceous hosts were planted at Caribou on June 2, 1915, in powdery-scab-infected soil in adjoining beds about 3 feet square, potatoes

having already been planted on three sides of the experimental plot. Of the 53 species planted, only 16 grew; but this is not surprising, in view of the fact that many were from tropical sections of the world, while the summer season at Caribou is distinctly temperate and short.

The solanaceous plants which became infected are:

Solanum warscewiczii.
Solanum haematoclodum.
Solanum mammosum.
Solanum marginatum.
Solanum ciliatum.
Solanum commersoni.
Lycopersicon esculentum.

Those which remained free from infection are:

Solanum nigrum.
Solanum mauritianum.
Solanum duplosumatum.
Solanum labelii.
Solanum heteracanthum.
Solanum seaforthianum.
Solanum laciniatum.
Solanum torvum.
Solanum sp.

As shown by the lists, infection resulted on 7 of the 16 species that grew, and all that grew were species of the genus Solanum except *Lycopersicon esculentum* (tomato).

In the case of *Solanum commersoni*, a tuber-bearing plant very closely related to *S. tuberosum*, the cultivated potato, 50 hills were planted and 46 grew. On August 20 six of these hills were dug and examined. Numerous galls, similar to those on the potato plant, were found on the roots, but the tubers were free from infection, as were also the tubers from the remaining hills when harvested, on October 10.

In the case of *Lycopersicon esculentum*, the seed was sown rather thick; consequently the plants, none of which grew to be more than a foot high, were crowded in the bed and the soil was a solid mass of roots. Infection was first found on August 20, at which time the galls were quite pronounced and were present on roots of all sizes (Pl. 9, fig. B, C). The galls were examined for evidence of immature spore balls; but, in marked contrast to the potato galls, in which immature spore balls were very evident, none were found. Even as late as September 24 the galls contained no spore balls. This is believed to be due to the fact that the host plant continued rapid growth until killed by frost; for as long as the growth of the host continues the fungus penetrates deeper into the tissues, without any marked tendency to form spore balls.

In the case of *Solanum warscewiczii*, a subtropical ornamental plant and probably a native of South America, 22 of the 66 plants growing in the bed were infected. Two very interesting points in the reaction of this host are the large size of the galls and their formation in a ring around the taproot (Pl. 9, fig. A). Although there seemed to be no difference in general vigor between the infected and noninfected plants, nevertheless this girdling of the taproot doubtless interferes materially with the natural processes of the plant. As in the case of *Lycopersicon*

esculentum, no spore balls could be found even after the plants had been killed by frost.

The other four hosts did not do as well as *Lycopersicon esculentum* and *Solanum warscewiczii*, but their root systems were quite extensive and showed numerous galls having superficial characteristics common to those in the potato and tomato. The phloem was vigorously attacked by the parasite, which caused the xylem and vessels to be twisted out of their normal course in many instances, and the cells of the phlœm were hypertrophied and contained a considerable quantity of starch grains.

The most significant fact brought out in these tests is that infection took place in the root system of the tomato, and this is important in view of the fact that the tomato is very extensively grown in the United States, and often on land used for potatoes. The roots are very generally infected and the distortions and malformations more conspicuous and destructive than those on the potato (Pl. 7, fig. C, D).

When *Spongospora subterranea* was found to thrive on hosts other than the potato, examinations were made of the root systems of weeds common in and about potato fields, including members of the Cruciferae, Labiatae, Scrophulariaceae, and many other families, and also of cultivated plants, such as clover, oats, wheat, and barley; but in no case were signs of infection found. No wild species of Solanum were found growing as a weed in the potato fields or in vacant lots near by.

HISTOLOGY OF THE GALLS

The galls on the roots of the potato are simple in structure and may be termed kataplasmatic galls (Pl. 7, fig. C, D). The fungus or exciting agent flourishes only in the phloem or meristematic tissue (Pl. 8, fig. A), as explained by Woronin (17) in the case of rootlets of cabbage seedlings. Occasionally amebæ are seen in the xylem, but they have never been found to be numerous or to show any signs of stimulating the cells to further growth. The plasmodium of the fungus enters the cell and causes marked hypertrophy, which distends the cell until it is from 80 to 140μ in length. Later this divides by cross walls until instead of one large cell there may be as many as six normal-sized cells, all of which have a nucleus and are surrounded by a goodly number of amebæ, which would indicate that there is some sort of reaction between these bodies. Occasionally, however, one or more of the amebæ are distantly removed from the host nucleus. As the gall enlarges, this hyperplastic growth of the phloem tissues often pushes the vascular system out of its normal position. The infected cells always contain numerous large and small starch grains, which do not wholly disappear until the spore balls are mature. Osborn (11) claimed that the fungus feeds on starch; but were this the case, such an abundant supply would not always be present. It seems clear, however, that the fungus stimulates the protoplast to produce

abundant starch. The amebæ finally bunch up around the host nucleus, which disappears before the spore ball is mature. The amebæ vary from 2.5 to 3μ in diameter, are uninucleate and spherical, and contain a single nucleus, which stains heavily with safranin (Pl. 8, fig. A). As in the case of the tuber, the diseased root cells differ from the healthy in that they show a large number of various-sized starch grains.

In *Solanum warscewiczii* the infection is likewise confined to the phloem and the infected cells occur in groups (Pl. 8, fig. B). These groups originate by continual division of one or more cells in both *L. esculentum* (Pl. 8, fig. C) and *S. warscewiczii* (Pl. 8, fig. B), which has been described by Nawaschin (10) for "clubroot" and called by him "Krankheitsherde." However, the starch grains are not as abundant nor as large in the cells of *S. warscewiczii* as in those of the potato or tomato; in fact, numerous infected cells without a trace of starch are often found. The host nucleus in the infected cells does not differ in size from those found in diseased cells, but the cells proper are increased in size and elongate and form the giant cells referred to by Kunkel (6) in the case of the potato. Such cells are also referred to in the case of Ostenfeldiella attacking *Diplanthera wrightii*. Two such giant cells from the potato partially surrounded by smaller healthy cells are shown in Plate 8, figure B. In one of these giant cells it will be noticed that there is only a single cross wall. In another case one of the cells after dividing into three cells showed early stages of another division. Each of the three cells measured approximately 22.3μ in diameter and was almost square, while the cells immediately surrounding these and belonging to the same histological tissue measured only 11.25μ. The amebæ do not differ in size from those in the tomato, and as in the case of that host are found grouped around the nucleus.

It will be noticed that spore balls were not produced on either the tomato or *Solanum warscewiczii*. This is due to the fact that the host plants had not reached maturity before they were killed by frost, the tomato being about 1 foot high and *S. warscewiczii* just blossoming. There can be no question, however, as to the causal agent in either case, as the amebæ in both were uninucleate and showed the same measurements, staining reaction, position in the host cell, and presence in the phloem, which reacts alike in all of the different hosts.

SPONGOSPORA SUBTERRANEA ON THE TUBER

The first evidence of Spongospora infection on the tubers consists of faint, brownish purple, fimbricate, discolored areas about the size of pinheads and resembling in outline very small bacterial colonies (Pl. 10, fig. B). These spots indicate that the causal organism has entered and destroyed the cells immediately under the epidermal layer. At this stage the infection is comparatively superficial; but in from six to eight days the spot may increase in diameter to ½ cm., lose its brownish color, and be replaced by a somewhat jellylike protuberance, consisting of metaplastic

tuber tissue filled with spore balls of the fungus. The diseased tissue gradually dies, disintegrates, and liberates the spore balls, the sorus at this time being mature. The earliest infections on the tuber usually occur about the stem end, but as the host matures pustules may develop about the eye end.

The symptoms do not always agree with the foregoing, but may vary in accordance with external conditions. For instance, it would seem that if a tuber were detached from the mother plant at the time infection is becoming visible and immediately placed in a moist chamber the development of the pustule should continue; but this is not the case, for while the infected area may increase a little, its development is seriously checked. Again, if the stems are cut off at the surface of the soil in the early stages of infection, the formation of a pustule is checked, but the discolored area may increase and the epidermis may be raised slightly, which symptoms suggest that the plasmodium continues to vegetate rather than to break up and form spore balls. Two rows from which the tops were removed on August 11, six days after the first infection was found on one of the plots, showed only 3 per cent infection, while the checks showed 24 per cent, but the former showed a reduction of about 75 per cent in yield. In like manner, if plants are attacked by lateblight shortly after infection with *Spongospora subterranea* begins to appear, further infection is stopped, and that which has already begun makes but little progress. This occurred in many fields in Maine during the season of 1915. The severe outbreak of lateblight checked the development of the potato crop and of powdery-scab. On the other hand, if the potato plant continues to grow after infection has once taken place and external conditions are favorable for the development of *S. subterranea*, the sori increase in depth and diameter (Pl. 10, fig. C), which suggests the canker stage of *S. subterranea* known in Ireland. The tuber shown in Plate 10, figure C, is the progeny of a plant that grew in the greenhouse and did not mature and die down until 152 days after planting, although its tubers showed infection after 76 days. The unusually large size of the sori in this case is attributed to the long growing period of the plant, which was about 40 days longer than usual. Such sori are common where potato plants are grown in wet, infected soil in the greenhouse and would doubtless result in the field if the crop had a longer growing season. In most potato-growing sections along the Atlantic seaboard only about 100 days are allowed, this being especially true in northern Maine.

These greenhouse experiments suggest that the absence of the canker stage in the United States may be due to the short growing period for the potato plant in districts in which *Spongospora subterranea* thrives.

A careful examination of material showing the canker stage as it exists in Ireland[1] (Pl. 10, fig. E) proved that it differs from the prevailing type of scab caused by *Spongospora subterranea* prevalent in this country.

[1] This material was obtained through the kindness of Dr. George H. Pethybridge.

The symptoms are not fully comparable with those produced in the greenhouse, although they had some points in common, as is shown in Plate 10, figures C, D, and E. The diseased surface of this material presented the appearance of having been gnawed and chewed by insects after *S. subterranea* had made its appearance. The wide variation in the symptoms of this disease emphasizes its dependence on environmental conditions.

The resemblance of the injury caused by the flea beetle to that caused by the sori of *Spongospora subterranea* is very striking, as evidenced by the fact that specimens showing the former were received from several pathologists with an inquiry in each case as to whether the injury was due to powdery-scab. There is no doubt that insects play a rôle in the destruction of the diseased portions of the potato plant. The injury probably caused by the flea beetle and other subterraneous larvæ (Pl. 10, fig. A) can be distinguished from that due to *S. subterranea* by the fact that in its earlier stages there is a minute central opening caused by the puncture of the insect, and in its later stages the affected area may show definite splitting, which, however, does not extend very deep into the tissues. Frequently the central portion of the raised area is crumbled, and it is at this stage that the trouble is most often confused with the open pustules of *S. subterranea*. At this stage also it shows another similarity to *S. subterranea*—that is, the sunken area immediately surrounding the sorus, which is due either to what is designated and described later in this paper as physical drying out (Pl. 11, fig. F, H) or to the action of the plasmodium (Pl. 11, fig. D).

In the course of the work the writers also received many specimens showing enlarged lenticels (Pl. 10, fig. F), resembling superficially the early stages of powdery-scab, with an inquiry in each case as to whether the injury was due to *Spongospora subterranea*. Intumescence associated with the lenticels develops when the tubers are held in moist chambers for 10 days. The proliferation of the cells below and around the lenticels at the end of that time resembles the protruding hyperplastic tissue of the sorus in early stages of formation. A microscopic examination and the identification of spore balls within the sorus, however, is the criterion for determining the disease, but, as will be pointed out in the following paragraphs, care should be taken not to confuse the spore form of other fungi with the spore balls of *S. subterranea*.

CONFUSION IN EARLIER WRITINGS DUE TO THE FUNGI ASSOCIATED WITH SPONGOSPORA SUBTERRANEA ON THE POTATO TUBER

Wallroth (15) and Berkeley (1) described fungi associated with a potato disease which bear a striking resemblance to the spore balls of *Spongospora subterranea*, and earlier investigators observed numerous fungus threads in the sori, all of which resulted in much confusion and error. The writers believe that the "bulbils" of a species of Papulospora

which they isolated from the sori of *S. subterranea* were largely responsible for this confusion and error.

Wallroth saw in the spore balls of *Spongospora subterranea* a striking resemblance to the smuts and accordingly described the organism as *Erysibe subterranea*. Berkeley (1) more than 20 years after Wallroth's observations published a drawing of two spore forms in connection with an article on the potato murrain and refers these two forms to the spores of *Tubercinia* sp. Fr., which cause a scab. The spore forms shown in Berkeley's drawings are certainly not the spore balls of *S. subterranea*, as the former show a distinct pedicel, but Berkeley's description of their effect on the tuber is highly suggestive of the latter fungus. The surface of the potato, he says, is "covered with pustules, which at length become cup-shaped and are powdered within with an olive-yellow meal consisting of the spores of a fungus." The drawings and description of the fungus create some doubt as to whether he referred to *S. subterranea*. In view of his illustration and statement that he saw the various stages of growth attached to flocci, it is possible he was working with material which in addition to being infected with *S. subterranea* was contaminated with the "bulbils" of *Papulospora* sp. or with the forms referred to by Horne (3)

The presence of fungus hyphæ in the sorus and the consequent hypothesis that there are two kinds of powdery-scab is shown by the following statement by Johnson (5, p. 172):

> In some Scotch material I examined, hyphæ were clearly present in the scab areas outside of the cellular tissue of the tuber, and though some could be accounted for as the mycelial hyphæ of Rhizoctonia scab there were others not so explicable. The hyphæ are swollen, septate, and branching; their contents abundant and granular. In some case chains or masses of spores may be seen arising from the protoplasmic contents of the hyphæ. Spongy spore-balls, very like those of Spongospora, arise, and ultimately the inclosing walls of the hyphæ disappear and leave the balls lying free. The more external balls lose their compactness and break up into single spores or small groups of spores, so that they form a finer powder than Spongospora, whose spore-balls remain intact to the end. The mode of origin of the spores is not unlike that met with in the *Ustilagineæ*, so far as they have been studied; and it thus appears as if . . . there are two kinds of potato scab characterized by powdery spore-balls, the one with a plasmodium—*Spongospora subterranea* Wallr.—the other a Hyphomycete, possibly one of the Ustilaginaceæ.

Horne (3, p. 377, 380) says:

> If spore-balls are present, they are frequently associated with the hyphæ of various fungi—the association is so close in some instances that it is difficult to convince oneself that the spore-balls are not the reproductive bodies of the fungus. . . . In late stages of the disease, and even in the powdery-scab stage, the spore-balls . . . are frequently intimately associated with the hyphæ of various fungi. The spore-balls appear sometimes to be attached to hyphæ, or hyphæ twine around them and link them together.

These statements are indeed significant and show that extreme care must be taken in identifying the spore balls of *Spongospora subterranea*,

which are never attached to hyphæ. Plate 12, fig. G, shows figures given by Horne of spores of fungi associated with *S. subterranea*, and referred by him to species of Verticillium and Stysanus, and also drawings of the "bulbils" (Pl. 12, fig. F) of *Papulospora* sp. found by the writers; the similarity between these is very striking.

As early as 1883 Eidam (2, p. 411–414) called attention to the similarity between a species of Papulospora and one of the smuts. It is the "bulbils" of *Papulospora* sp. that bear such a striking resemblance to the smuts and also to the spore balls of *Spongospora subterranea*. Hotson (4) published a paper that treats in part of the genus Papulospora and includes a good description of the "bulbil." In this paper he says (p. 299):

> Lastly, among the structures which bear a striking resemblance to bulbils, the peculiar spore-balls of *Spongospora subterranea* (Vallr.) Johnson, should be mentioned; which although they might readily be taken for a species of Papulospora, have been shown to belong to the life-cycle of one of the Mycetozoa.

In view of the recent isolation of *Papulospora* sp. by one of the writers from tubers affected with *Spongospora subterranea* this statement is very significant. A culture of this species of Papulospora submitted to Dr. J. W. Hotson was identified by him as *Papulospora coprophila* (Zukal) Hotson. All inoculations on the potato tuber with this fungus gave negative results.

CONTROL MEASURES

Although it has been known for more than 50 years that *Spongospora subterranea* occurs in most northern and central countries of Europe, very little effort has been concentrated on its control; and such studies as have been made apply only to local conditions in Ireland, where cultural, soil, and weather conditions are markedly different from those in the potato-growing sections of the United States. As soon, therefore, as it was found that the disease had become established in some of the leading potato-growing sections of this country, the study of its control became imperative and was undertaken by the writers along four lines: (1) Early harvesting, (2) seed treatment, (3) varietal response, and (4) soil treatment.

EARLY HARVESTING

That *Spongospora subterranea* develops on the tubers only after they are partially mature has already been shown. Although carefully sought for during the entire season of 1914 in the plots in which infected seed was planted, no infections were found on the crop until August 20; and not until about three weeks later did it become common or conspicuous. The first case of infection in the warehouse at Caribou was found on September 12 by the Maine Potato Inspection Service, which employed

about 50 inspectors. By that time 12 per cent of the crop had been dug and much of it doubtless thereby saved from infection. Were infection to appear as late every season, early harvesting would be a very simple and effective means of avoiding the disease.

The results obtained in 1914 suggested early harvesting as a means of wholly avoiding the disease where the crop was growing on land known to be infected. To test this four rows 16 rods long were planted on infected land in 1915. Two of these rows were harvested on August 15, when about two-thirds mature, or about two weeks earlier than usual, the harvesting season here beginning about September 1 and extending to October 10. The remaining two rows were dug on October 10. The two rows dug in August produced 323 pounds; of this lot 153 tubers were infected. The two dug in October produced 412 pounds, of which 167 tubers were infected. These figures show that practically all the infection in 1915 made its appearance before August 15, and that from the standpoint of control nothing was gained by harvesting on August 15, while 89 pounds of potatoes were lost in the two rows harvested at that time. However, had infection taken place as late in 1915 as it did in 1914, the crop on a given farm might have been harvested before it became infected; consequently it is believed that this line of attack has not been exhausted and that it has greater possibilities than the results obtained in 1915 indicate.

SEED TREATMENT

In the spring of 1914 an experiment to control the disease by disinfecting the seed potatoes badly infected with *Spongospora subterranea* was begun, Green Mountain, the variety commonly grown in northern Maine, being used. The seed was treated as shown in Table VI.

TABLE VI.—*Results of experiments in controlling powdery-scab by seed treatment in 1914*

Plot No.	Treatment.	Hills.				
		Average weight.	Total number.	Number sound.	Number infected.	Percentage of infection.
		Pounds.				
1	Check, infected seed	1.39	200	63	137	68.0
2	Seed wet and rolled in sulphur	1.48	152	85	67	44.0
3	Formaldehyde (1:30), 1½ hours	1.27	164	138	26	15.8
4	Atomic sulphur (5 per cent), 1½ hours	1.15	202	148	54	26.7
5	Formaldehyde (2:30), 1½ hours	1.04	136	116	20	14.7
6	Mercuric chlorid (2:15), 1½ hours	1.05	189	184	4	2.0
7	Check, infected seed	.....	164	92	70	42.0
8	Copper sulphate (5 per cent), 1½ hours	.94	142	137	5	3.5
9	Mercuric chlorid (4:15), 53°–54° C., 5 minutes	.93	75	70	5	6.6
10	Formaldehyde (2:30), 46°–50° C., 5 minutes	.90	55	54	1	1.8
11	Mercuric chlorid (4:15), 44°–45° C., 5 minutes	1.14	75	69	6	8.0
12	Check, healthy seed	1.25	200	198	2	1.0

TABLE VI.—*Results of experiments in controlling powdery-scab by seed treatment in 1914*—Continued

Plot No.	Treatment.	Sound tubers.		Infected tubers.		
		Number.	Weight.	Number.	Weight.	Per cent.
			Pounds.		*Pounds.*	
1	Check, infected seed	1,790	222	531	56.5	22.87
2	Seed wet and rolled in sulphur	1,613	200	221	26.0	12.00
3	Formaldehyde (1:30), 1½ hours	1,613	200	56	9.0	3.30
4	Atomic sulphur (5 per cent), 1½ hours	1,750	217	118	16.5	6.30
5	Formaldehyde (2:30), 1½ hours	1,097	136	42	6.5	3.68
6	Mercuric chlorid (2:15), 1½ hours	1,600	198.5	6	.75	.37
7	Check, infected seed	1,260	155	170	2.4	11.80
8	Copper sulphate (5 per cent), 1½ hours	1,068	132.5	9	1.0	.83
9	Mercuric chlorid (4:15), 53°–54° C., 5 minutes	556	69	7	.75	1.24
10	Formaldehyde (2:30), 46°–50° C., 5 minutes	397	49.25	2	.5	.50
11	Mercuric chlorid (4:15), 44°–45° C., 5 minutes	681	84.5	12	1.5	1.73
12	Check, healthy seed	1,640	204	4	.5	.20

After treatment the seed was spread out to dry on a laboratory floor which had been previously covered with paper. When dry, the potatoes were cut and placed at once in 1-peck sacks that had been previously soaked for three hours in a 1 per cent solution of copper sulphate or in sacks that had never contained potatoes. In this way they were kept apart from other potatoes and other sources of infection until planted, June 13. The seed used in the clean checks was carefully selected from several barrels believed to be free from powdery-scab, treated with the usual strength of mercuric chlorid, dried, cut, and bagged in the manner just described. Except in the case of clean check tubers, which were wholly free, each seed piece bore a considerable number of powdery-scab pustules.

The plot selected for the experiment was an old orchard which sloped gently toward the east and which had been in sod the preceding 10 years. The soil had been ridged up toward the apple trees; and consequently the ground was somewhat uneven. The soil was a black gravelly loam, but too wet and heavy for ideal potato land. A complete commercial fertilizer was used at the rate of 1,200 pounds per acre. Because of the irregularity of the ground and shading from the orchard trees, no attempt was made to record the germination of the seed or the development of the potato vines during the season. The relative position of the plots with reference to each other was the same as in the table, and the crop was harvested on October 15.

As shown by Table VI, the progeny in the plots adjoining plot 11, with the exception of one tuber in each of two hills, was free from infection by *Spongospora subterranea*, which showed that the land was not infected previous to the planting in 1914. Notwithstanding this nominal infection in the healthy check tubers, the amount of powdery-scab that developed on the various plots would represent the relative efficiency of the various treatments if other conditions were equal, which was not the case,

for, as already stated, the land was uneven on account of the ridging, and plots 1 and 2 were at the edge of the orchard and had more sunlight and consequently gave a larger average yield per hill.

The hill was considered a unit, each being harvested separately. The tubers were examined individually, being washed before examination if not clean when taken out of the soil, and a record made of the number of infected and the number of sound tubers in each hill and also of the gross weight of each.

As shown by Table VI, some of the progeny of the checks planted with diseased seed gave from 42 to 68 per cent of infection, the variation depending doubtless on the soil and water conditions in different parts of the field.

In drawing conclusions from the results given in Table VI it must be borne in mind that in each case only a comparatively small number of hills were used and that variation in the soil and moisture contents materially influenced the results. Notwithstanding these facts, however, it is perfectly obvious from the tests that several chemicals have a deleterious effect on the development of *Spongospora subterranea*, the most active being mercuric chlorid and formaldehyde, hot solutions being more effective than cold.

During the season of 1915 the most promising of the experiments made in 1914 were duplicated, the land used being cleared in the spring and put in condition for planting. This land was much lower than that used in 1914 and not so well drained. The soil was a rather heavy gray silt with considerable humus at the surface. The soil of parts of this plot was of the type on which much of the infection in northern Maine occurs. The land was planted by hand on June 10, the seed being handled in the same way as that used in the experiment of 1914. The arrangement of the plots with regard to each other and the results obtained when harvested on October 10 are given in Table VII.

The most striking result of this experiment is the infection of the control plots planted with healthy seed. One of these check plots was on each side of the field, which consisted of about one-fourth of an acre, and one in the middle, the former being numbered 1 and 9 and the latter, in which there were four rows, 18. Plots 1 and 9 received the same treatment as the plots used in the experiment in 1914, but plot 18 received special care in preparation and planting; moreover, the seed used in this plot was rigidly selected, inspected, and treated with double-strength mercuric chlorid. Everything with which it came in contact until planted was also disinfected with mercuric chlorid.

TABLE VII.—*Results of experiments in controlling powdery-scab by seed treatment in 1915*

Plot No.	Treatment.	Hills.				Tubers.			
		Total number.	Number sound.	Number infected.	Percentage of infection.	Total number.	Number sound.	Number infected.	Percentage of infection.
1	Check, clean seed	56	47	9	16.0	282	263	19	6.7
2	Check, infected cut seed	64	22	42	65.6	442	349	93	21.0
3	Formaldehyde (2:30), 1½ hours	66	49	17	25.7	392	364	28	7.1
4	Mercuric chlorid+ethyl alcohol (2:15), 1½ hours; 1,000 c. c. of alcohol in 3½ gallons of water	72	59	13	18.0	363	346	17	4.6
5	Check, infected seed	73	26	47	64.3	416	297	119	28.6
6	Atomic sulphur (5 per cent), 1½ hours.	84	39	45	53.5	469	388	81	17.2
7	Mercuric chlorid (4:15), 50° C., 5 minutes	65	55	10	15.3	332	321	11	3.3
8	Check, infected cut seed	56	9	47	83.9	428	304	124	28.9
9	Check, clean seed	73	61	12	16.4	449	431	18	4.0
10	Wet and rolled in sulphur	57	37	20	35.0	366	332	34	9.2
11	Formaldehyde (1:30), 1½ hours	93	82	11	11.8	486	474	12	2.4
12	Check, infected cut seed	71	49	22	31.0	374	329	45	12.0
13	Formaldehyde (2:30), 50° C., 5 minutes	78	72	6	7.7	366	359	7	1.9
14	Mercuric chlorid (1:15), 1½ hours	69	63	6	8.7	386	380	6	1.5
15	Copper sulphate (5 per cent), 1½ hours	49	40	9	18.3	327	300	27	8.2
16	Check, infected whole seed	88	19	69	78.4	780	506	274	35.1
17	Mercuric chlorid (2:15), 1½ hours	100	85	15	15.0	391	370	21	5.3
18	Check, clean seed	138	124	14	10.1	643	629	14	2.1

As will be seen in Table VII, 10.1 per cent of the progeny of the hills in plot 18 became infected, and as in all probability the seed was free from infection and the disease is not indigenous to virgin land, the question arises as to the source of the infection. In the case of the healthy control plots the probable source of infection was the adjoining infected controls, soil water, animal life, and cultural methods being the probable agencies by which the disease was spread. Considering the results from each plot individually and in relation to those in adjoining plots, it would seem reasonable to believe that in the case of the healthy checks adjoining those planted with untreated seed, the latter served as centers of infection. In 8 out of 10 cases more infection occurred in the rows next to the untreated checks than in the rows farther away.

Although the infection of the healthy checks diminishes the value of the seed-treatment experiment, it serves to emphasize the infectiousness of the disease and to some extent indicates the rate and means of spread where conditions are favorable for its growth. In the light of the developments on the healthy plots in 1915 it is easily understood that the two cases of infection in the clean control plots in 1914 were doubtless carried from the infected to the healthy controls.

As in the case of the experiments in 1914, those in 1915 show, in addition to the spread of the organism in the soil, that seed disinfection has a beneficial effect in diminishing the amount of infection and that treatment with mercuric chlorid and formaldehyde are the most effective,

treatments with a hot solution of these for a short time being more efficient than with a cold solution for a longer time.

The relative efficiency of the various disinfectants, according to the data obtained for the two years, is shown in Table VIII.

TABLE VIII.—*Average results in controlling powdery-scab obtained from different disinfectants used in the tests of 1914 and 1915*

Treatment.	Average percentage of hills infected.	Average percentage of tubers infected.
Formaldehyde (2:30), 46°–50° C., 5 minutes	4.75	1.20
Check, clean seed	7.58	2.23
Mercuric chlorid (4:15), 44°–45° C., 5 minutes	8.00	1.73
Mercuric chlorid (2:15), 1½ hours	8.50	2.83
Mercuric chlorid (1:15), 1½ hours	8.70	1.50
Copper sulphate (5 per cent), 1½ hours	10.90	4.50
Mercuric chlorid (4:15), 50°–54° C., 5 minutes	10.95	2.27
Formaldehyde (1:30), 1½ hours	13.80	2.85
Mercuric chlorid + ethyl alcohol (2:15), 1½ hours; 1,000 c. c. of alcohol in 3½ gallons of water	18.00	4.60
Formaldehyde (2:30), 1½ hours	20.20	5.39
Cut and rolled in sulphur	39.50	10.60
Atomic sulphur (5 per cent), 1½ hours	40.10	11.75
Checks, powdery-scab seed	64.60	25.12

VARIETAL RESPONSE

For a number of years the Office of Cotton and Truck Disease Investigations has carried on experiments on varietal susceptibility of potatoes to the lateblight fungus (*Phytophthora infestans*) and in this connection has made an extensive collection of European and American varieties reputed to be more or less resistant to lateblight. The study of powdery-scab also suggested the selection of resistant varieties as a possible means of control, and accordingly it was decided to use the above collection in the experimental plots infected with powdery-scab. In addition to this collection of standard varieties, a collection of seedlings also was used in the experiment.[1]

A piece of ground with a uniform soil type and infected with *Spongospora subterranea* was selected and 25 hills each of the different varieties of seedlings were planted, the Green Mountain variety being planted in alternate hills as controls. Table VIII gives the varieties tested and the results of the tests, including the percentage ratio of infection in the varieties tested and the checks.

[1] These seedlings, which have not yet been distributed to farmers and seedsmen, were obtained through the courtesy of Prof. William Stuart, of Horticultural and Pomological Investigations, Bureau of Plant Industry, by whom they were developed.

TABLE IX.—*Results of tests of potatoes for resistance to powdery-scab in 1915*

Variety.	Tubers.			Control tubers (Green Mountain).			Infection[a] in terms of p. ct. var. ×100 p. ct. G. M.
	Number planted.	Number infected.	Percentage of infection.	Number planted.	Number infected.	Percentage of infection.	
Eldorado	54	0	0	98	5	4.8	0
Farys	67	0	0	54	12	18.1	0
Prof. Wohltman	60	0	0	53	19	26.3	0
Senator	215	0	0	159	9	5.3	0
Ursus	178	2	1.1	46	15	24.6	4.473
Pearl	256	2	0.7	65	9	12.1	5.785
Aldona	194	4	2.0	44	21	32.3	6.192
Gryf	452	21	4.4	24	9	27.2	16.176
Bonar	93	9	8.8	26	14	35.0	25.143
Cimbals	68	3	4.2	63	9	12.5	33.600
Constantia							
Gracya	323	46	12.4	17	7	29.1	42.612
Gedymin	215	18	7.7	103	17	14.1	54.610
Prof. Wohltman	56	39	41.0	54	39	41.8	98.086
Kalif	193	188	49.3	34	34	50.0	98.600
Topaz	88	12	12.0	144	14	8.8	136.364
Soliman	147	61	29.6	52	11	17.4	170.115

[a] In order to get a direct comparison between the different varieties, the percentage of infection in any variety was divided by the percentage of infection of the Green Mountain variety planted in the same row and the result multiplied by 100.

As shown in Table IX, 4 of the varieties tested escaped the disease, but none of the checks were entirely free, the percentage of infected tubers in the latter varying from 4.8 to 50 per cent. Out of the 16 rows, 3 showed over 15 per cent infection in the case of the varieties tested and 10 in the case of the checks, and the latter fact naturally raised the question as to why the percentage of infection varied so in the checks, all of which were planted to the same variety.

While it may be that the soil was not generally infected or that conditions in small isolated spots were unfavorable for the development of the disease, the fact that there was often a wide difference in the amount of infection in alternate hills of the control and the other variety in the same row is significant. A striking example of this latter is shown in the row planted to Farys and the row of Ursus, the former showing no infection and the latter 1.1 per cent of infection, while the Green Mountain, the control variety planted in alternate hills in these rows, showed 18.1 and 26.6 per cent of infection, respectively. Another interesting reaction was that in the Soliman variety, which showed 29 per cent, while its check showed only 11 per cent of infection.

Thirty different selections of seedlings were planted in infected soil, and the checks in this case also were planted with the Green Mountain variety in alternate hills in each row. Table X gives the details of the experiment.

TABLE X.—*Results of tests of potato seedlings for resistance to powdery-scab in 1915*

Collection No.	Seedlings.			Control tubers (Green Mountain).			Infection in terms of p. ct. seed × 100 p. ct. G. M.
	Number planted.	Number infected.	Percentage of infection.	Number planted.	Number infected.	Percentage of infection.	
1357	79	0	0	130	18	12.1	0
1488	101	0	0	44	30	40.5	0
992	98	0	0	52	2	3.7	0
2892	125	0	0	58	5	7.9	0
628	99	0	0	36	2	5.2	0
2315	59	0	0	98	9	8.4	0
2387	128	0	0	42	4	8.7	0
2193	90	1	1.1	21	6	22.2	4.955
1522	129	3	2.2	26	12	31.5	6.984
1124	160	7	4.2	34	26	43.3	9.700
22402	151	2	1.3	148	23	13.4	9.702
2426	141	3	3.0	63	13	17.1	11.696
4259	119	5	4.0	43	20	31.7	12.618
1429	174	7	3.8	28	5	15.1	15.166
1055	113	18	13.7	15	14	48.2	28.423
4755	112	19	14.5	72	52	41.9	24.606
3760	113	3	2.5	54	4	6.9	36.232
2294	116	1	0.8	103	2	1.9	42.105
2950	126	37	22.7	27	19	41.3	54.964
1212	109	1	0.9	194	3	1.5	60.000
1034	144	21	12.7	137	32	18.9	67.196
2870	171	20	10.4	53	8	13.1	79.389
13660	105	21	16.6	42	11	20.7	80.193
4927	48	14	22.5	79	24	23.3	96.567
4227	213	88	29.2	80	27	25.2	115.873
9681	36	5	12.2	95	9	8.6	141.861
15284	89	72	44.7	25	10	28.5	156.842
14329	107	117	52.2	108	53	32.9	158.663
13896	110	12	9.7	46	3	6.1	159.016
1449	90	114	55.8	109	22	16.8	332.143

As will be seen by a comparison of Tables IX and X, the response to the disease was very similar in the standard varieties and the seedlings, except that the extremes of infection were greater in the latter, ranging from 0 to 55.8. Seven of the seedlings showed no infection, while the checks were infected in every case, the infection varying from 1.5 to 48.2; or in the rows of the nine cases referred to, from 3.7 to 40.5. Although the tubers of these nine varieties were free, the roots showed a goodly number of galls, which indicates either that the roots and tubers do not resist the disease to the same degree or that the tubers merely escaped the disease.

As already explained, infection in 1915 took place only for about two weeks during the growing season—that is, between August 1 and 15—and in case the tubers had not set by this time or had not reached a susceptible stage, infection was avoided. It was also observed that, so far as the amount of infection is concerned, it made no difference whether the variety was early or late. That there is a close correlation between

the development of the cork cambium and susceptibility is not improbable, and this is strongly indicated by the fact that probably 90 per cent of the pustules on the infected tubers from the experimental plots were about the stem ends rather than the eye ends. While this preponderance of infection about the stem end was true in 1915 on the experimental plots, the condition in this respect in other fields was not determined, and no such preponderance was noted during the previous season, when infection occurred much later, or between August 20 and about September 5. A critical examination of some of the varieties and of the seedlings attacked showed that not only the percentage of tubers infected but also the macroscopic character of the sori differ.

Different varieties of potatoes respond differently to the attacks of the disease, as shown in Plate A, which represents various kinds of sori. It was possible to arrange a series from mature sori, varying from the size of pin pricks to that of 1 or 2 cm. as they occur on such standard varieties as Green Mountain and Irish Cobbler. The reaction of the host showing the smaller sori differed markedly from that of the host showing the larger sori. In some varieties the sorus attains a considerable size before it bursts, in others it breaks open very early, while in still others the infected area corresponding to the sorus shows only discoloration and gives the impression that the plasmodium is spread throughout this discolored area, but at no point does it cause sufficient proliferation of the host cells to form a definite open sorus.

The data regarding the response of the different varieties lead the writers to believe that the response is not due entirely to resistance but rather to the fact that the tubers of certain varieties have escaped infection, and furthermore that a variety which will show much less infection than the Green Mountain may be found.

SOIL TREATMENT

Five acres on one of the farms first cleared in the vicinity of Caribou and said to have been under cultivation for at least 30 years were used in 1915 for the study of effects of soil type and soil treatment on the disease. Because of its close proximity to the village, this land has been cropped rather heavily to potatoes during the previous 15 years.

Topographically the tract occupies the position of a high glacial river terrace sloping from west to east. Along the northeast side and in part forming the northern boundary is a well-marked drainage way or ravine in which considerable erosion has taken place, leaving the soil exceedingly stony and with less fine earth than elsewhere in the field. With the exception of this and a small area at the southwest corner, the slopes are not steep and there is little or no noticeable erosion. In the central part of the field the slope appears inconsiderable, but the instrumental determination showed not less than 5 feet elevation in 100 horizontal feet, a

slope sufficient for the rapid drainage of surface waters. On the tract were found certain fairly well marked differences of color, texture, and other physical characteristics of the soil. These soils, which are here designated by a set of arbitrary numbers, are as follows:[1]

Soil 1.0.—The surface is grayish brown to ashy gray, depending on the moisture content. The texture is silty loam, fairly friable when dry, but somewhat plastic when wet. Numerous small stones and gravel occur in the soil and subsoil, the latter being a light ashy-gray or mottled gray and brown and rather heavy silty loam. The drainage of this soil is sufficient for surface run-off, but appears to be deficient in the subsoil, probably due to seepage of water from the higher lying upland. This type comprises the greater area of the tract.

Soil 1.1.—The surface of this soil is light brown and is underlain by a subsoil which is gray and much like that of No. 1.0. The texture is about the same as that of the foregoing type, though the structure of the surface is more open and friable, stone and gravel are not so conspicuous a feature, the surface is more sloping, and the subsoil drainage is freer.

Soil 1.2.—The color of the surface is slightly darker than that of No. 1.0, though the subsoil is about the same and the texture is a silty loam. In the area in the northeastern corner of the tract the content of small stone and gravel is extremely high, so that the amount of interstitial soil material is considerably reduced. The two small areas on the southeastern corner of the tract are less stony, the percentage being about the same as in soil 1.0. The texture of the subsoil is rather finer than that of the surface and more compact, the color being a dark grayish brown or gray slightly mottled with brown to the depth of 3 feet or more. The soil occupies relative depressions along natural drainage ways, and in the larger area there is considerable erosion. While the surface drainage seems adequate, there is so much seepage apparently that the internal drainage is actually poorer than in soil 1.0.

Soil 2.0.—This soil is a medium- to light-brown silty loam and contains considerable gravel of a smaller size generally than is common to the other types. The subsoil is reddish brown to yellowish brown, with a somewhat larger amount of small gravel than in the surface. There is no mottling, oxidation being quite uniform in both soil and subsoil. This type differs from all other soils in that there is no gray mottling in the subsoil. The topography is abrupt or rolling and both surface drainage and subdrainage are free.

Figure 1[2] shows the distribution of the soil types on the portion of the field used.

[1] The description of soils here given was very courteously contributed by Mr. J. E. Lapham, Scientist in Soil Survey, Bureau of Soils.

[2] This map was made by Dr. Oswald Schreiner and Messrs. J. E. Lapham and H. L. Westover, of the Bureau of Soils, through whose kindness it is published. It was of great value in interpreting results of the experiments.

As there will be seen from this map, there are five different types of soil, which occur very irregularly in both the upper and the lower block. The diagram superimposed on the map shows the arrangement of the plots, the distribution of *Spongospora subterranea*, the percentage by weight of the progeny infected with the disease, and the yield per acre. The seed used was of the Green Mountain variety and was carefully selected and treated with the usual strength of mercuric chlorid. The plots, which were numbered 1 to 15, inclusive, received treatment as follows:[1]

1.—Sodium nitrate, 20 pounds.
2.—Control, with commercial fertilizer, 150 pounds.
3.—Old horse manure, 2,400 pounds.
4.—Control; no treatment.
5.—New horse manure, 2,400 pounds.
6.—Phosphoric acid, 24 pounds.
7.—Ammonium sulphate, 20 pounds, and phosphoric acid, 24 pounds.
9.—Potassium chlorid, 30 pounds.
10.—Ammonium sulphate, 20 pounds.
11.—Flowers of sulphur in drills, 90 pounds.
12.—Flowers of sulphur broadcast, 90 pounds.
13.—Calcium carbonate, 300 pounds.
1b.—Sodium nitrate, 20 pounds.
2b.—Control with commercial fertilizer, 150 pounds.
4b.—Control; no treatment.
7b.—Ammonium sulphate, 20 pounds, and phosphoric acid, 24 pounds.
13b.—Ammonium sulphate, 20 pounds.
14b.—Flowers of sulphur, 90 pounds.
15b.—Phosphoric acid, 24 pounds.

As will be seen from the foregoing outline of treatments, the effect of the common commercial-fertilizer ingredients, as well as of some of the well-known soil disinfectants, were studied on the plots either with or without commercial fertilizer. The treatments in all the plots were applied with the potato planter and at time of planting, except in the case of those marked "b," which were applied on August 12, a few days after the first infections were noted, and each treatment was duplicated on what was known as the upper and lower blocks.

Seven of the control plots received at the rate of 1,500 pounds of commercial fertilizer per acre, or 150 pounds each, and an equal number received no treatment. In the former the percentage of infection ranged from 15.1 to 35.4 and averaged 24.7 per cent, while the latter showed a greater variation, ranging from 4.9 to 36.5 and averaging 23.4, or a total average of 24.05 for the 14 control plots. None of the treated plots gave as high a percentage of infection as the average of the control plots, and those treated with sulphur at the rate of 900 pounds per acre gave the lowest percentage, the average of the four thus treated being 8.7 per cent.

[1] All plots marked "b" received treatment on August 12.

Lime at the rate of 3,000 pounds per acre increased the amount of infection 13.2 per cent over its nearest control in the upper block, but diminished it as compared with the control plot in the lower block, and in this case also reduced the yield 28.3 bushels per acre. The difference in the reaction of lime on the two blocks, however, was probably due to the difference in the soil types in the two cases, which are plainly shown in figure 1.

When the crop was harvested, every other row in each plot was dug with the machine and the tubers examined and sorted before being picked up. A stake was placed at the last case of infection in each row, which was determined and plotted as shown by the shaded area in figure 1. In the

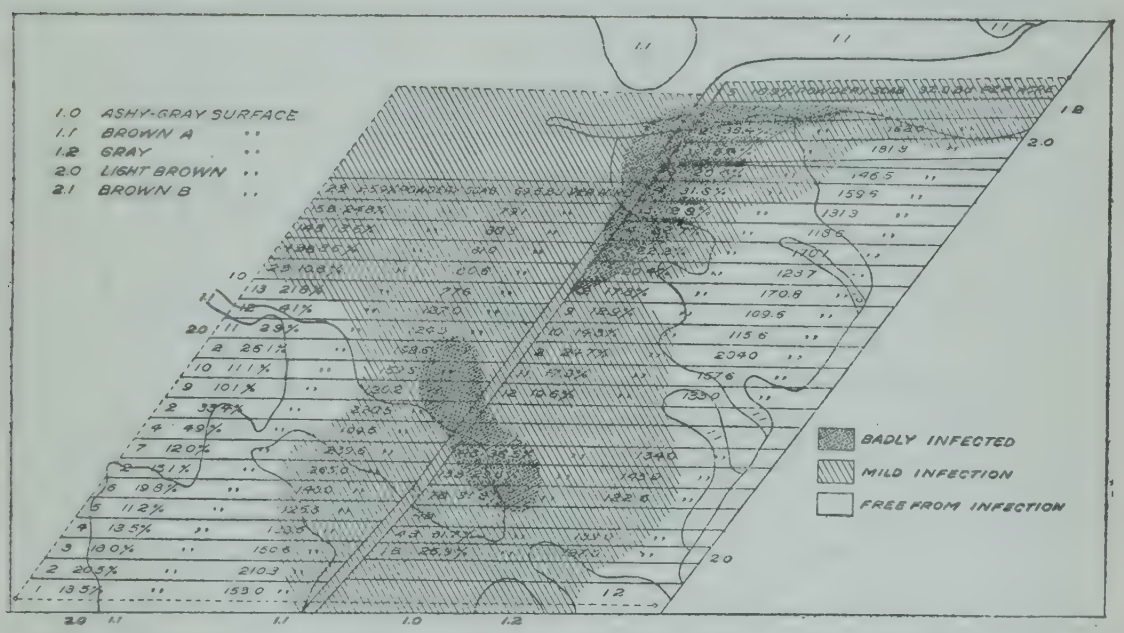

FIG. 1.—Map of the experimental plots at Caribou, Me., showing their arrangement, distribution of *Spongospora subterranea*, percentage by weight of the progeny infected with the disease, and the yield per acre.

lower block the boundary line between the infected and the noninfected portions of the plots was more difficult to mark, the infections being few and scattered south of the lightly-shaded area in the block, especially in the area between plots 7 and 3. The fact that soil 1.1 is partly infected in the corner is doubtless due to seepage from soil 1.0 lying above it.

The significant point brought out in this study is the close relationship of the development of *Spongospora subterranea* to the Washburn silt-loam type of soil, which is marked "1.0" on the map. It was repeatedly observed that where infection was bad the soil had a grayish surface, which showed plainly that it was of the 1.0 silt-loam type. Exceptions to this have been found, but in general there is a close correlation.

Table XI shows the comparative value of the treatments on plots similar in other respects:

TABLE XI.—*Comparative effect of different soil treatments on powdery-scab and on the yield of potatoes*

Plat No.	Treatment.	Percentage of powdery-scab.	Bushels per acre.
1	Sodium nitrate	16.95	138.3
2	Control with commercial fertilizer	24.7	205.8
3	Old horse manure	18.2	165.9
4	Control with no fertilizer	23.8	123.2
5	New horse manure	11.05	108.8
6	Acid phosphate	20.1	143.2
7	Ammonium sulphate and acid phosphate	17.1	204.8
9	Potassium chlorid	11.5	114.9
10	Ammonium sulphate	12.7	137.5
11	Sulphur drill	10.1	140.9
12	Sulphur broadcast	7.35	130.0
13	Calcium carbonate	17.3	104.4

As shown by Table XI, ammonium sulphate and acid phosphate gave nearly the same yield as the checks fertilized, and in addition diminished the amount of infection by *Spongospora subterranea* 7.6 per cent. In the plots treated with potassium chlorid there was less infection than in any of the plats receiving other fertilizer ingredients, or only 11.5 per cent. This is attributed to the slow growth of the plants in the early part of the season and their continued growth until killed by frost. The potassium chlorid apparently prolonged the growing season, and it may be that the crop in a measure escaped the infection period, which, as already shown, was in August.

While these experiments extended through only one season and consequently only tentative conclusions can be drawn, they demonstrated that sulphur at the rate of 900 pounds per acre applied broadcast reduced the amount of infection by *Spongospora subterranea*, and all of the fertilizer ingredients tested reduced the amount of infection from 5 to 12 per cent when applied alone.

DRY-ROT ASSOCIATED WITH SPONGOSPORA SUBTERRANEA

Although *Spongospora subterranea* has been known in Europe since early in the forties of the last century, no mention has been made of a dryrot commonly associated with and following the disease. This rot differs markedly from the many rots which have already been described and which are common to the potato tuber, and a discussion of it necessarily involves the description of several types of rot not heretofore distinguished and a study of their causes. This is the rot to which the senior writer in his brief mention (7) of the shriveling and shrinking

which occur around some of the sori, and the importance of the part played in this connection by the wound parasites which enter through the injury caused by *S. subterranea*. In view of the fact that the rot was first found associated with *S. subterranea* and is most common on potatoes infected with the disease caused by that organism, it seems desirable to call it "powdery-scab dryrot."

HISTORY AND DISTRIBUTION

Powdery-scab dryrot was first observed by the senior writer on potatoes infected with *Spongospora subterranea* collected in New Brunswick, Canada, in the fall of 1913, and held in storage in Washington, D. C. Later it was collected in Aroostook County, Me., and in the infected section of New York State.

Although first found in America, the rot is not confined to this country. A typical case of it was found in a shipment from Ireland by Mr. H. B. Shaw, Pathological Inspector of the Port of New York. About a bushel of the infected tubers collected and sent by Mr. Shaw to one of the writers for examination as to the causal organism showed pronounced discoloration and shrinkage and later on a rot. In the fall of 1913 three 1-barrel sacks were taken at the port of New York from a shipment of potatoes from the Netherlands which showed a considerable percentage of infection by *Spongospora subterranea* and was shipped to Washington, where the diseased tubers were separated from the healthy ones and both lots placed in storage. When these were examined two months later, 21 per cent showed shrinkage and rotten spots.

A dryrot has been found on infected tubers collected in Chile, South America, but in this case the rot was not marked, as in the case of the European tubers, and may well have been accentuated by the long period of transit under poor storage conditions. Be this as it may, however, it is perfectly clear that to some extent at least a dryrot following infection by *Spongospora subterranea* develops on potatoes, no matter where they are grown.

PREVALENCE OF AND LOSSES FROM POWDERY-SCAB DRYROT

Powdery-scab dryrot develops on potatoes after they have been held in storage for some time. It is accelerated by poor storage conditions, but even in good storage from 30 to 75 per cent of the tubers become partially or wholly decayed and consequently worthless for seed or table use. Morse (9) refers very briefly to a rot connected with *Spongospora subterranea* which is doubtless the same as the one under consideration here. This rot, he states, develops on potatoes held in good storage and is hastened when infected potatoes are subjected to ordinary room temperature for a few days.

While it is not an uncommon thing to find powdery-scab-infected tubers entirely decayed, the rot generally occurs in spots. The spots vary from 1 to 10 cm. in diameter. They may be only slight depressions in the superficial layers, or they may extend into the center of the tuber. In this respect powdery-scab differs markedly from the scab caused by *Oospora scabies*, with which it may be easily confused before the dryrot sets in. The rotting of the tubers following infection by *S. subterranea* not only distinguishes this disease from that caused from *O. scabies*, but emphasizes its more destructive nature.

Some idea of the prevalence and destructiveness of powdery-scab dry-rot can be formed from the percentage of this rot on tubers grown during the season of 1913 infected by *Spongospora subterranea*. Infected potatoes were collected from individual growers at different points in Aroostook County between May 2 and June 15, 1914. These were washed and separated into two lots, those showing dryrot and those free from the rot but infected with powdery-scab. Later the lots were weighed, and the percentage proportion by weight of those showing the rot is given in Table XII, together with other data regarding the tubers used.

TABLE XII.—*Percentage of powdery-scab dryrot on potatoes of the 1913 crop infected with Spongospora subterranea*

Date of collection.	Place of collection.	Variety.	Quantity collected.	Percentage of powdery-scab dry-rot.
			Bushels.	
May 2	Patten, Me.	Green Mountain	6	73
May 5	Presque Isle, Me.	Irish Cobbler	3	30
May 5	Caribou, Me.	Green Mountain	8	33
May 21	Patten, Me.	do	5	67
June 10	Caribou, Me.	do	12	45
June 15	Ashland, Me.	Irish Cobbler, Green Mountain.	5	35

As will be seen from Table XII, nearly 50 per cent of the tubers showed the dry-rot stage, the percentage of rot on the two varieties ranging from 30 to 73.

Although limited, these observations indicate that the Green Mountain is more severely attacked than the Irish Cobbler. This may be due to varietal differences, but it seems more logical to believe that it is due to the fact that the Green Mountain is a later-maturing variety.

The rot was most prevalent on the tubers infected with the scabby stage of *Spongospora subterranea*, and this explains the low percentage found on the two lots collected at Presque Isle and Caribou on May 5. A very high percentage of the tubers badly infected with the scabby stage showed the dryrot in some form. Not only does the scabby stage of powdery-scab mar and deface the tuber and render it objectionable in

the market, but it may be followed by a rot which will render the tuber worthless for either table or seed purposes.

Naturally the question arose as to whether tubers infected with *Spongospora subterranea* harvested while immature will rot more than such tubers harvested when mature; and in order to get light on this phase of the subject, experiments were carried on with the crop of 1914. About two weeks before the harvest season, or on September 9, 91 tubers were dug from the experimental plots and examined. Out of the total number, 67 showed various stages of infection, ranging from a few small, immature pustules to a generous sprinkling with sori, while 24 were free from the disease. After this the diseased tubers were placed in one sack and the healthy tubers in another, and both sacks were placed in a potato-storage cellar, such as is commonly used in northern Maine. On June 15, 1915, both sacks were examined, and it was found that of the 61 tubers in the sack containing the infected potatoes 31 were two-thirds or wholly rotten, 4 had rotten spots from 2 to 4 cm. in diameter, and 32 had rotten spots from 1 to 2 cm. in diameter. With one exception, which was perfectly sound, the sori apparently having corked over perfectly and prevented the entrance of fungi, all the tubers generally infected with sori of *S. subterranea* were rotten. The 32 tubers with small dryrot spots consisted of those which showed few small sori of *S. subterranea*. Of the 24 tubers in the sack containing the healthy potatoes, 3 were two-thirds or wholly rotten, 1 had a rotten spot 2 cm. in diameter, and 20 were sound, except in the case of 2 which were infected with common scab.

In another case 1 bushel of powdery-scab-infected tubers was collected from a field at the beginning of harvest, or on September 24. At this time the tubers were not fully mature, and the sori in most cases had not ruptured the epidermis; but the badly infected specimens showed brownish purple areas. These potatoes, together with others, were placed in storage. When examined for the development of the rot, on June 10, 1915, 63.9 per cent of the tubers were found to be decayed two-thirds or more, and the collection was in such a condition that it could scarcely be handled. In every case the sound tubers were those which showed but little powdery-scab.

From these results it is evident that powdery-scab dryrot becomes much worse on potatoes harvested early than on those harvested after the tubers are fully mature.

In another case 620 tubers, the progeny of 110 hills in a field infected with powdery-scab, were harvested on November 7, 1914, the potatoes being dug by hand. Each tuber was carefully examined, and it was found that 63 per cent of the lot by weight was infected and that the progeny of only two hills was free from the powdery-scab. The healthy tubers, numbering 295, and the tubers infected with *S. subterranea*, numbering 325, were put in separate sacks and placed in storage on November 9, 1914. When taken out and examined, on June 14, 1915,

only 32 of the sound tubers, or about 11 per cent, showed signs of decay, while 188 of the infected tubers, or about 58 per cent, showed the rot. About 3 per cent of the former and about 58 per cent of the latter were worthless for table use.

Although interesting, the data gathered from the crop of 1913 was not definite, owing to the fact that the conditions under which the potatoes were grown and stored were unknown. More exact data were obtained from the crop of 1914, which was grown on 15 acres of land located in a field in which 75 per cent of the hills had produced tubers infected with *Spongospora subterranea*. From this 15-acre plot 16 barrels were collected on October 7, as they were being harvested, and placed in good storage at Caribou the following day. The amount of infection shown by these potatoes varied from only a few sori to enough literally to cover the tuber. On June 15, 1915, parts of 7 of the 16 barrels were sorted over, and the potatoes showing powdery-scab but no rot were separated from those showing rot. Table XIV gives the results of this study.

TABLE XIV.—*Occurrence of powdery-scab dryrot on the crop of 1914*

Barrel No.	Number of bushels examined.	Number of tubers free from powdery-scab.	Number of tubers showing powdery-scab.	Percentage of tubers showing powdery-scab.
1	1	70	124	63.9
2	2½	170	173	50.1
3	2½	190	368	65.9
4	1	92	124	59.6
5	1½	238	149	40.1
6	1½	38	175	55.9

As will be seen from Table XIV, from 38 to 65.9 per cent of the tubers infected with powdery-scab rotted to such an extent as to be unfit for table use, and this notwithstanding the fact that these potatoes were harvested when fully mature and placed in good storage at once, as already stated.

SYMPTOMS OF POWDERY-SCAB DRYROT

The relation of *Spongospora subterranea* to powdery-scab dryrot is in a way comparable to the rot which follows *Phytophthora infestans*. *S. subterranea* causes little rot, but it leaves an open wound, through which wound parasites may enter. It is well known that *P. infestans* causes little rot unless it is followed by bacteria and wound parasites.

The symptoms of the dryrot following infection by *Spongospora subterranea* may vary greatly, according to the time of year, storage conditions, state of the tuber when harvested, and the stage of development of the sorus when the infected tuber is removed from the soil. In

general, however, the symptoms may be divided into three groups: (1) Desiccation or loss of moisture from the wound caused by *S. subterranea*, (2) those caused by the germination of spore balls in the bottom of the sorus and the formation of the plasmodium which destroys the adjoining cells, and (3) those caused by the entrance of wound parasites into the sori. Often, however, all three causes work together, and in many cases the fungi begin their work after some desiccation and plasmodium injury have set in. It should also be borne in mind that dryrot does not occur about every sorus of a tuber infected with *S. subterranea*. A tuber may be well sprinkled with sori and yet show no dryrot; or the rot may appear about a few of the sori, this latter being true in the case of infected tubers harvested both when immature and when quite ripe and filled with countless spore balls.

Powdery-scab dryrot due to the desiccation of the tissues adjoining the sorus results in discoloration, shriveling, and shrinkage. When infested tubers are harvested, the sori are virtually open wounds, and when placed in warm, dry storage the temperatures incident to which often prevail in early fall, desiccation of the living cells bordering on the wound takes place. When the storage temperature drops with the advance of the season, this type of dryrot is retarded. It can be induced readily, however, by taking tubers from storage and holding them for a fortnight at ordinary room temperature. The cause of the desiccation of the cells about a sorus is readily seen in sections of the sori made before and after shrinkage takes place. Plate 12, figure A, shows the condition in the bottom of a sorus before there is any appreciable amount of desiccation. It should be especially noted that no cork has formed in the bottom of the pit, the adjoining living cells being protected by the dead débris and spore balls of the sorus. By referring to Plate 14, figure A, it will be seen that abundant cork cells are formed in connection with a sorus of common scab. This explains why dryrot is very seldom or never associated with this disease.

A comparison of figures A in Plates 12 and 14 shows clearly why desiccation might take place in one case and not in the other.

The condition of a sorus after some dryrot due to desiccation has taken place is shown in Plate 12, figure B. When the material was dehydrated, the spore balls of the fungus were washed out. It is especially interesting to note the number of dead cells that are still partly intact. There are also signs of the formation of some wound cork. Where this was found, the underlying cells were still alive. There was no evidence in this sorus of a plasmodium, such as occurs in connection with dryrot due to plasmodium injury. The type of dryrot due to desiccation never causes serious damage, but it does further mar the appearance of tubers already injured by the sori of *Spongospora subterranea*.

The second type of dryrot is that caused by the plasmodium of *Spongospora subterranea*. The spore balls in the bottom of the sorus germinate

and form plasmodia, which move about in the immediate vicinity of the sorus and destroy the host cells. This type of dryrot has recently been described by Kunkel (6). The destruction of the cells is followed by a brownish discoloration and shrinkage, which in the early stages is often most conspicuous on one side of the sorus, but later surrounds the pustule and a small depressed area appears (Pl. 11, fig. D). The plasmodium, which has never been found except in the parenchyma, works largely in the superficial tissues, producing a dry, hard spot from ½ to 1 cm. in diameter and ¼ to ½ cm. in depth. Isolations made from this type of rot often give a variety of fungi, most of which are saprophytes; but in some of these isolations wound parasites exist and probably find such injured spots excellent points at which to begin the complete destruction of the tuber.

The entrance of wound parasites through the open sori marks the beginning of the third and most destructive type of powdery-scab dryrot (Pl. 11, fig. B, C, E). In the bottom of many of the sori there is little or no wound cork (Pl. 12, fig. A, B), and the pit is filled with dead tissues and numerous spore balls in the same way as open cavities in the tuber are filled with small masses of culture media on which fungi naturally flourish.

The most common of the wound parasites found associated with the early stages of rot is a species of Phoma, producing brownish to gray lesions (Pl. A and Pl. 11, fig. A). As these lesions progress they become more sunken, darker, and often hard and bony; and when removed, which can often be easily done, they leave a clean and smooth cavity in the tissues of the tuber. The shape and texture of the spots removed give the impression of a button; hence the name "button-rot," by which the trouble is known among farmers. Later stages of the lesions (Pl. 13, fig. A, B) vary from 2 mm. to 5 cm. in diameter and often reach a depth of 2 to 4 cm. (Pl. 11, fig. C). The diseased tissues are sharply defined on the surface, where the pustules are numerous and where infection may take place through each of the pustules; in this way large areas may become discolored and later depressed (Pl. 11, fig. E).

Often, after the above-described lesions have formed, other wound-rot and decay organisms enter, in which case the symptoms are somewhat confusing (Pl. 11, fig. B, C, E). The more common of these organisms are *Fusarium coeruleum* (Lib.) Sacc., *F. discolor*, var. *sulphureum* Schlect., and various bacteria. The symptoms of each of these are typical of the particular species, as described by Wollenweber (16). These fungi have been repeatedly isolated and identified from single-spore cultures as well as from typical rots produced in potato tubers artificially inoculated. Generally when any of these wound-rot organisms are present, the lesion is soggy and less firm to the touch, and its surface is often cracked and broken.

ISOLATION STUDIES

During the past three years a large number of isolations were made from the dryrot lesions, and a considerable number of parasitic and saprophytic organisms, including species of Coniothyrium, Ramularia, Periola, Fusarium, Phoma, Rhizoctonia, Vermicularia, Papulospora, and Bacteria, were studied as regards their relation to dryrot.

A type of spot, which at first had brownish to gray lesions and later became hard and dark (Pl. 13, fig. A, B), was found to be very common, not only in connection with *Spongospora subterranea* but also on tubers free from this disease, the senior writer (7) having found it in Maine before the presence of *S. subterranea* had been reported.

When not associated with any of the fungi above mentioned, the rot caused by the species of Phoma under consideration in section is slate-colored, dry, and powdery; but when other fungi are associated the tissues of the tuber show cavities or chambers and the color is characteristic of the mycelium or spores of the fungus present—that is, white and blue in the case of *Fusarium coeruleum*, light green or sulphur-colored in the case of *F. discolor*, var. *sulphureum*, and dark brown when *Papulospora coprophila* is the secondary saprophyte. The following studies indicate the distinctive characteristics of the rot caused by the species of Phoma under consideration.

Twenty tubers showing typical symptoms were selected for these studies. These were washed and immersed in mercuric chlorid (1:1,000) for 10 minutes, after which the surface tissue was peeled off and plantings made from the newly exposed tissue. In the majority of cases the plantings gave a pure culture of a species of Phoma. The isolations were made from button-shaped spots associated with and apart from *Spongospora subterranea*, and similar results were obtained in each case.

In order to get more accurate data regarding the association of the fungus with the lesion, a more detailed study was made. Twenty tubers having typical lesions were selected on May 17, 1915, an effort being made to secure tubers in which no other wound fungi had entered. The lesions, which varied from 6 to 25 mm. in diameter, were firm to the touch, dark gray, and had the appearance of typical button-shaped spots. The isolation from each tuber was made on a separate plate, and 4 plantings were made on each plate, or a total of 80. These were examined on May 20, and the number and kind of colonies found on each are shown in Table XIV.

As shown by Table XIV, the 80 plantings produced 6 bacterial and 46 fungus colonies. Microscopic examination showed that the latter included at least three different groups of fungi: One containing 2 colonies, one 38 colonies, and one 6 colonies.

In order to check up the identity of these colonies, 2 transfers were made from each of the 46 colonies, to test tubes of potato hard agar and

of sterilized sweet-clover stems. The results obtained confirmed the examinations made direct from the plates. The 38 colonies on both the agar and the sterilized sweet clover in all the test tubes proved to be a species of Phoma. Inoculations were also made with one or more of the fungi from each group, which showed that only the fungus included in the 38 colonies was pathogenic.

TABLE XIV.—*Results of isolations from tubers showing typical lesions caused by the rot of Phoma sp.*

Plate No.	Number of colonies growing on plate.	Number of growths produced on plate.	
		Bacterial.	Fungus.
1	4	0	4
2	0	0	0
3	0	0	0
4	0	0	0
5	0	0	0
6	4	0	4
7	2	0	2
8	3	0	3
9	1	0	1
10	4	2	2
11	2	0	2
12	2	0	2
13	4	0	4
14	4	0	4
15	4	0	4
16	4	0	4
17	3	0	3
18	3	3	0
19	4	0	4
20	4	1	3
Total	52	6	46

INOCULATION OF TUBERS WITH PHOMA SP.

As the fungus was obtained from the tuber, a series of inoculations was made on tubers, which were selected, washed, and immersed in mercuric chlorid (1:1,000) for 10 minutes, or in some cases in 85 per cent alcohol and burned over the surface. By means of a flamed scalpel the tuber was cut to a depth of 1 to 3 cm., a piece of a pure culture from a transfer 4 days old inserted, and the point of inoculation marked with india ink, after which the potatoes were placed in a moist chamber at ordinary room temperatures. At the end of four days the first indications of the disease became apparent. Ten days after inoculation the lesions reached 6 mm. in diameter. The diseased spots developed until they were from 12 to 25 mm. in diameter, but after this made little or no progress. The control tubers, treated in a similar manner but with no fungus inserted in the wound, remained healthy throughout the entire experiment. The writers have

never been able to produce larger lesions, which is significant, as in nature larger lesions are rarely found unless other wound parasites have entered. A tuber from one series of inoculations in which lesions are only 6 mm. in diameter are shown in Plate 13, figure E, and a series which produced much larger lesions in Plate 13, figure C, and for comparison tubers naturally infected are shown in Plate 13, figures A and B, and Plate 11, figure A. A tuber injured but not inoculated is also shown in Plate 13, figure D.

Table XV gives the results of a series of inoculations made during the course of the work.

TABLE XV.—*Results of inoculating potato tubers with Phoma sp. in 1915*

Date of inoculation.	Number of inoculations.	Percentage of infection.	Condition of control.
March 26	18	88	Healthy.
April 3	18	100	Do.
April 13	6	66	Do.
April 23	24	90	Do.
May 1	6	66⅔	Do.
May 10	9	66⅔	Do.
May 15	8	50	Do.
May 27	10	90	Do.

In the series of inoculations made on May 1 new potatoes were used. The fungus was able to produce very slight infection on these new potatoes, but the lesion never exceeded 3 mm. in diameter. The tubers inoculated May 10 showed only a slight infection. These tubers were held in the moist chamber until May 25, when they were examined. The infected area at this time measured 3 mm. in diameter, and in one inoculation scattered pycnidia could be seen with the naked eye and were as abundant near the edge of the spot as in the center. Reisolations were made on poured plates of potato agar from the inoculations made on March 26, April 3, and May 27, and in every case a pure culture of a fungus, macroscopically and microscopically identical with the culture used for inoculation, was obtained. Plate 14, fig. B, shows a section of one of the inoculated tubers. Most of the mycelium of *Phoma tuberosa* is beneath the epidermal cells. This mat later gives rise to the pycnidia, which grow towards the surface of the tuber.

CULTURAL CHARACTERISTICS

Tests made with the fungus on a number of media showed that it grows well on sterilized sweet-clover (*Melilotus alba*) stems, potato hard agar, potato cylinders, corn meal, and Beyerinck's agar. The luxuriance of its growth on all of these leaves no doubt that it is able to grow

on practically all of the common media. Since its growth does not differ in any essential characteristic from that of other species of Phoma, only the following brief cultural characters need to be mentioned: On sweet-clover stems the growth is fluffy, profuse, and white, turning gray with age; the pycnidial development is good, especially when little moisture is in the tube; the pycnospores often ooze to the surface. On potato hard agar its growth is profuse; the mycelial growth becomes evident within three or four days, is whitish at first, and at the end of a week begins to darken to the characteristic gray of *Phoma* spp.; the pycnidial development is very scarce. On potato cylinders the growth is fluffy and white, turning to gray within eight or nine days, the entire culture turning dark after the development of numerous pycnidia. On corn meal there is a profuse mycelial growth, at first light gray, turning darker with age; numerous pycnidia. On Beyerinck's agar the mycelial growth is scarce and white, and this medium is especially favorable for the production of pycnidial bodies (Pl. 12, fig. C, D).

By actual measurements it was found that there is no difference in size between the pycnospores from the host and those produced in culture, those from both sources, with occasional exceptions, varying from 3.7 to 6.00μ in length and 1.8 to 3.7μ in width (Pl. 12, fig. E).

TAXONOMY OF THE FUNGUS

Saccardo lists four species of Phoma (14) as occurring on *Solanum tuberosum: Phoma nebulosa* (Pers.) Mont., *P. eupyrena*, *P. solani* Cook and Harkn., and *P. solanicola* Prill. and Delacr. The original description of *P. solanicola* (13) states that it was found on the stems, but it was impossible to determine from the literature whether the three others were associated with the aerial part of the plant or with the tuber. The measurements for each of these four species and for the one under discussion are given in Table XVI.

TABLE XVI.—*Size of different species of Phoma occurring on Solanum tuberosum*

Species.	Size of pycnidia.	Size of spore.
	μ	μ
Phoma nebulosa	135–145 × 110–115	7.5–3
Phoma eupyrena	250 ("¼ mm.")	4–1½
Phoma solani	Minute.	7–8 × 1¾–2
Phoma solanicola	Minute.	6–7 × 4
Phoma sp. under consideration	90–160 × 80–160	3.78–6.10 × 1.8–3.7

Unfortunately it was impossible to obtain authentic material of the four species described. The original descriptions are too meager to enable one to identify a species with any degree of accuracy, but they show that in size of the spores the species differ markedly from the *Phoma* sp. under

consideration. These meager descriptions and difference in size of spores, coupled with the pathogenicity of this fungus on the tuber, led the writers to designate the organism "*Phoma tuberosa*, n. sp."

Phoma tuberosa, n. sp.

Lesions on tubers of *Solanum tuberosum;* brownish to dark gray or black; 6 to 25 mm. in diameter; sunken, membraneous, with an irregular and sharply defined margin. Pycnidia black, generally scattered over entire surface, subcuticular, irregular, subglobose to spherical, majority provided with a single well-defined ostiole, sometimes breaking at several points for exudation of spore mass, varying in size from 80 to 160 by 90 to 160μ. When placed in water the pycnospores are seen to ooze out in a shiny string, which soon breaks up into the individual spores. Pycnospores 1-celled, hyalin, subglobose, 3.7 to 6 by 1.8 to 3.7μ. Hyphæ septate, dark brown in the tissues of the host. A definite stroma absent.

Habitat.—Wound parasite on tubers of *Solanum tuberosum* often associated with the sori of *Spongospora subterranea*. First found as a storage-rot in Maine.

SUMMARY

(1) *Spongospora subterranea* exists in six different potato-growing sections of the United States, all northern except one.

(2) No infections resulted on the progeny of powdery-scab-infected seed potatoes planted in 15 different localities along the Atlantic seaboard. However, 8 lots of soil out of 12 shipped from as many of these localities to northern Maine and planted with infected seed produced a crop showing powdery-scab.

(3) Periods of damp, rainy, and cloudy weather, coupled with poor drainage, favor the development of *S. subterranea*.

(4) Infection develops earlier on the roots than on the tubers. In 1915 in northern Maine 57 days elapsed between planting of infected tubers in virgin soil and the first signs of root infection. Infection on the tubers appears about the stem end first. All underground portions of the potato plant may become infected with *S. subterranea*. Galls are often very numerous on the root system of potato plants growing in infected soil, while the tubers are absolutely free from infection; hence, a clean root system is the criterion for determining the absence of the disease.

(5) It is not unusual to find parts of fields in northern Maine in which some of the progeny of over 90 per cent of the hills are infected with powdery-scab. Several cases were found in which from 50 to 75 per cent of the 1914 crop was infected. It was found that *S. subterranea* may be spread when infected tubers are planted in virgin soil. Cultural practices and soil water are probably the most important agents in spreading the disease.

(6) Besides the potato, there are seven other solanaceous hosts of *S. subterranea*, including the tomato, as determined by the writers. The disease manifests itself on these hosts in the form of large destructive galls on the roots, these being fully as injurious as those on the potato

plant. The histology of the galls on all the hosts is very similar and has many points in common with *Plasmodiophora brassiceae* on cabbage.

(7) The absence of the canker stage of *S. subterranea* in the United States may be due to the short growing period afforded the potato crop in infected districts.

(8) Flea-beetle injury, intumescence associated with the lenticels, and certain forms of common scab on the tuber are often mistaken for stages of *S. subterranea.*

(9) Among the saprophytic fungi found associated with the sori of *S. subterranea* is a species of Papulospora. The "bulbils" of the latter are strikingly similar to the spore balls of the former fungus, and this similarity may account for the confusion in earlier writings as to its identity on the potato tuber.

(10) A study of early harvesting, seed treatment, varietal response, and soil treatment as control measures for the disease was made. This suggests that (a) early harvesting may be beneficial certain seasons in Maine, but can not be relied on every year; (b) seed treatment with certain chemicals will reduce the disease, this being especially true of mercuric chlorid and formaldehyde, the hot solutions for short periods being probably as efficient as the cold for longer periods; (c) certain varieties may escape infection; this may be due not to disease resistance but to differences in development at the time infection is most likely to take place; (d) the possibility of finding a resistant variety has not yet been exhausted; (e) no soil treatment will eradicate the disease, but sulphur at the rate of 900 pounds per acre applied broadcast reduces the amount of infection by *S. subterranea.*

(11) Several types of dryrot follow *S. subterranea.* These, designated according to cause, are desiccation, plasmodium, and wound-parasite injury. The percentage of these secondary rots as found in nature in infected tubers varied from 30 to 73.

(12) There is a close relation between certain soil types and the development of fungus. (See fig. 1.) From the type of soil and its drainage it is possible to predict what the development of the disease will be in any particular field.

(13) The dryrot due to a species of Phoma and other wound parasites is the most serious of the rots. After a comparison with earlier descriptions of various species of Phoma on the potato, the writers designated the species here discussed as "*Phoma tuberosa*, n. sp."

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PLATE A

Spongospora subterranea and *Phoma tuberosa* on *Solanum tuberosum:*

Fig. 1–5.—*Spongospora subterranea* as found on different varieties of the Irish potato. Figure 2 shows the sori on the Green Mountain variety. The sori shown in figures 1, 3, 4, and 5 are from seedling varieties not yet distributed.

Fig. 6, 7.—Stages in the development of dryrot caused by *Phoma tuberosa*. Figure 6 shows a very early stage and figure 7 a stage commonly found in April in storage houses.

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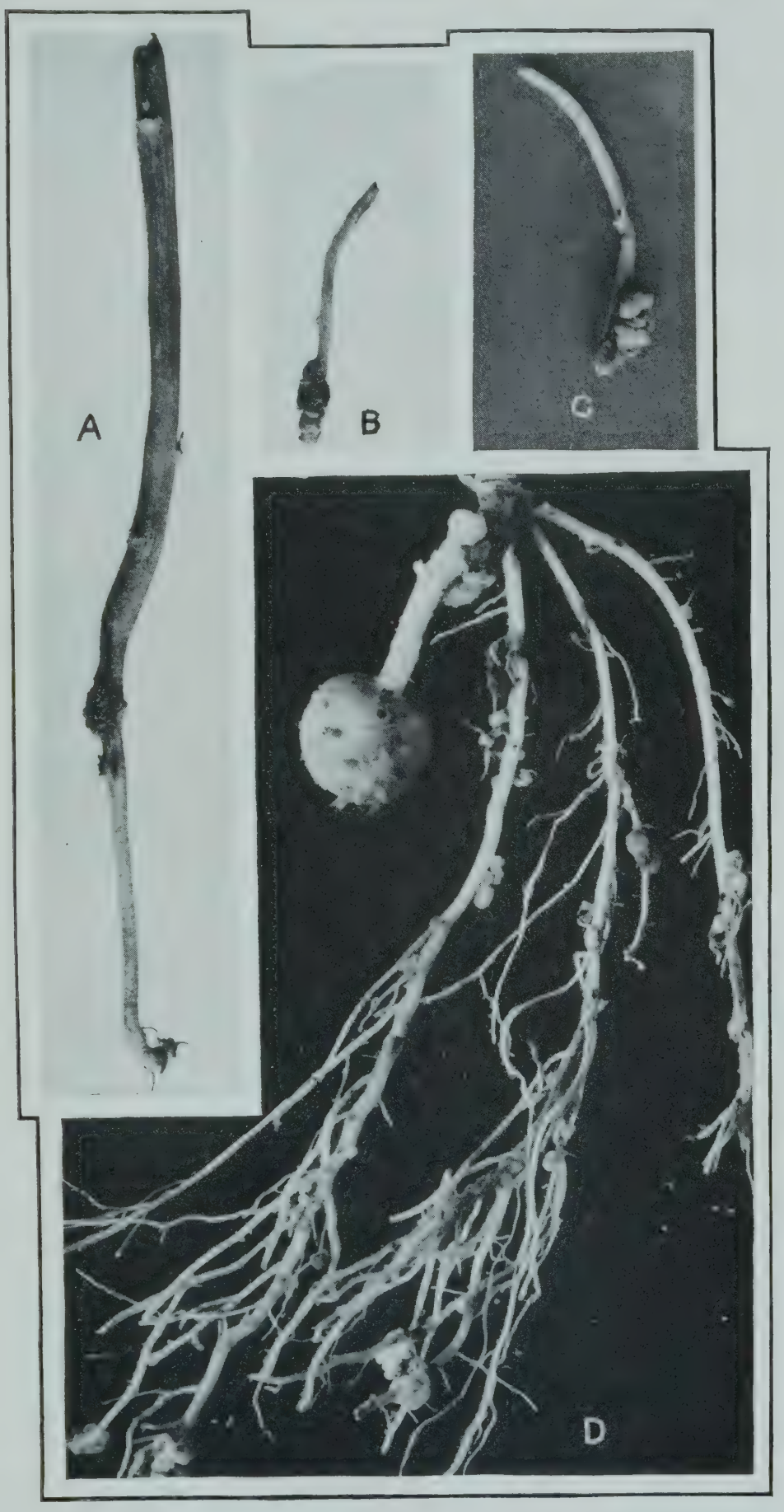
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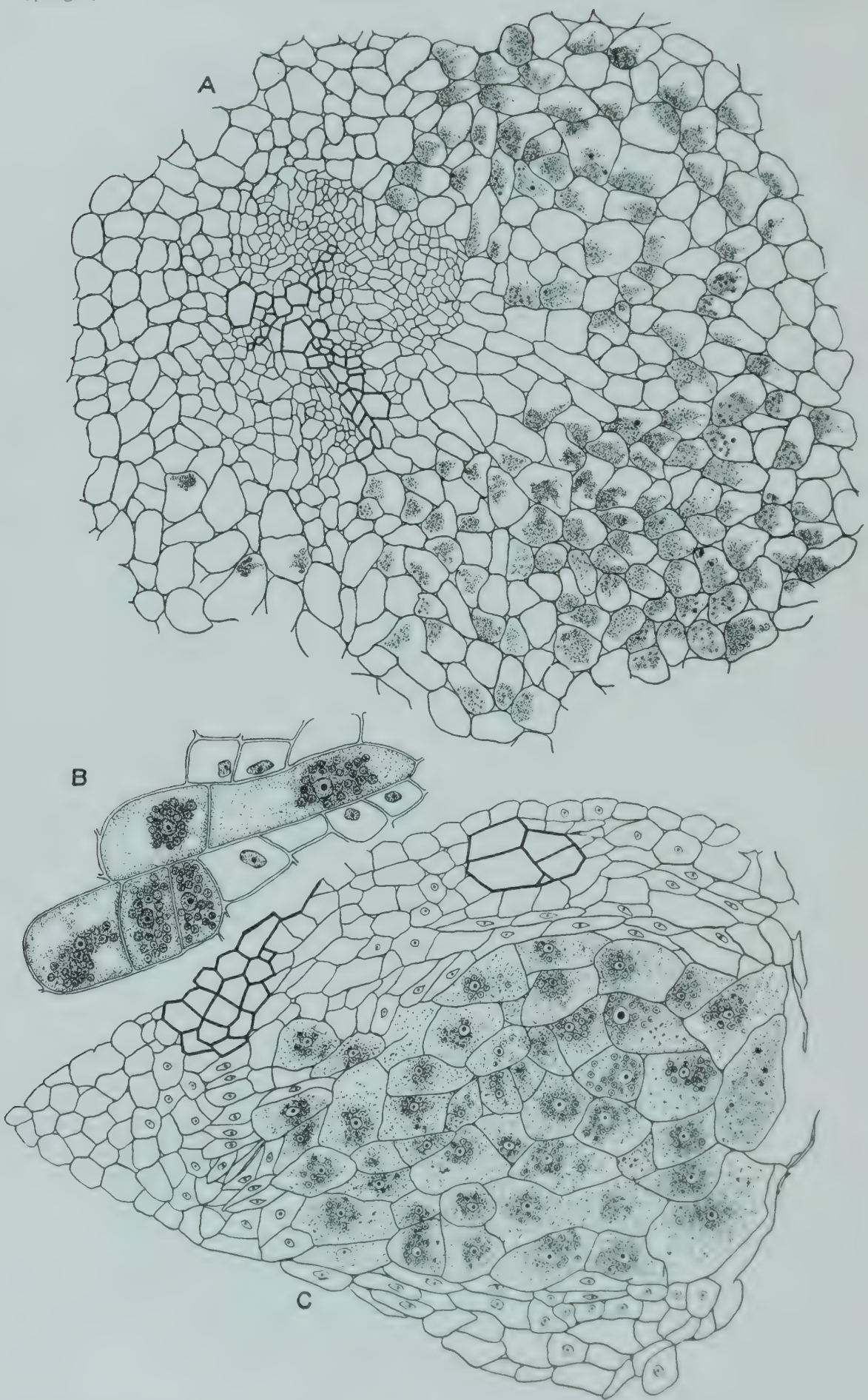
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PLATE 8

Spongospora subterranea in the roots of various hosts (fixed in Flemming's solution and stained with triple stain):

Fig. A.—Section through a potato root affected with *Spongospora subterranea*. The portion to the left is healthy, while that to the right shows the increase in number and size of the host cells. Amebæ in the cells are very numerous. × 110.

Fig. B.—Several cells from *Solanum warscewiczii*, showing the formation of "giant cells" and their division into daughter cells. Note the tendency of the amebæ to cluster around the host nucleus. × 2,600.

Fig. C.—Section through a tomato root (*Lycopersicon esculentum*, showing effects of infection by *Spongospora subterranea*. Note the abnormal increase in number and size of the cells. The parasite is confined to the cortex, being entirely absent from the xylem. The amebæ are numerous and generally clustered around the host nucleus. × 325.

PLATE 9

Spongospora subterranea on the roots of various hosts:

Fig. A.—Galls caused by *Spongospora subterranea* on the roots of *Solanum warscewiczii*. In this plant the galls have a tendency to girdle the root.

Fig. B, C.—Galls caused by *Spongospora subterranea* formed on the roots of the tomato. These galls, owing to the age and size of the plant, show a variety of forms.

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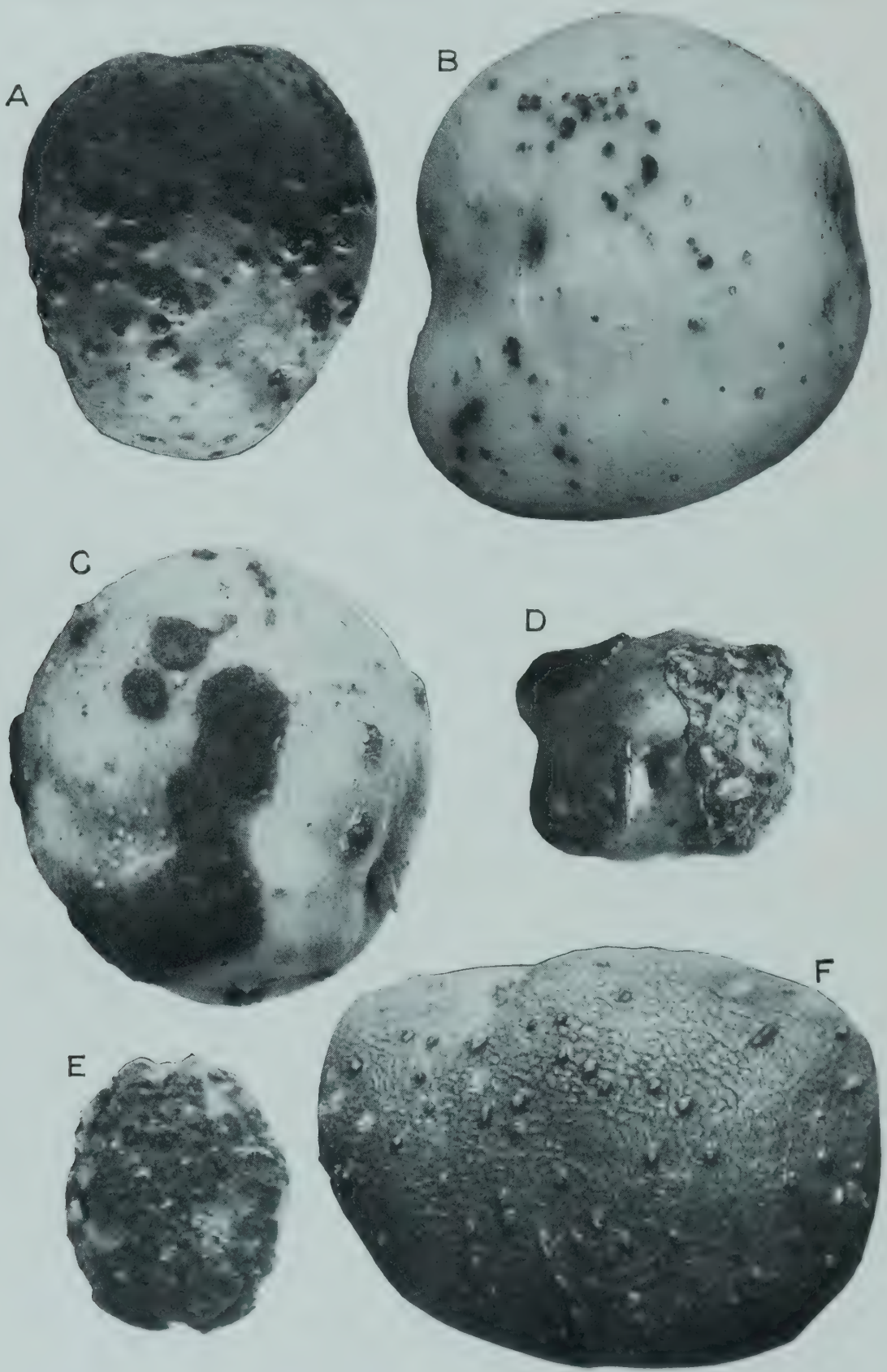
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PLATE 10

Injuries caused by *Spongospora subterranea* and other agencies:

Fig. A.—Tuber showing the effect of flea-beetle injury. Note the similarity of this injury and the young sori of *Spongospora subterranea*. After being placed in storage a shrinkage occurs around the sori-like injuries similar to the desiccation injury around the sori of *Spongospora subterranea*.

Fig. B.—Tuber showing a very early stage of infection by *Spongospora subterranea*. The points of infection show brown, fimbriate colonies. This condition is found in potatoes at time of harvesting. Spore balls are absent.

Fig. C, D.—Tubers grown in infected soil in the greenhouse under exceptionally moist conditions and allowed a long growing season. These lesions resemble very materially the cankerous stage.

Fig. E.—A potato from Ireland showing the cankerous stage. The powdery material is almost gone from the tuber, which appears to have been eaten by insects.

Fig. F.—A tuber showing enlargement of the lenticels. This tuber was kept in a moist chamber for 10 days. Similar enlargements are often found in Florida-grown potatoes, and have also been seen in Delaware and New Jersey. Such conditions are often mistaken for the early stages of infection caused by *Spongospora subterranea*.

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PLATE 11

Dryrots associated with *Spongospora subterranea:*

Fig. A.—A potato tuber showing natural infection with *Phoma* sp. The color of the spot is much darker than that of the healthy tissues. No pycnidia are present as yet.

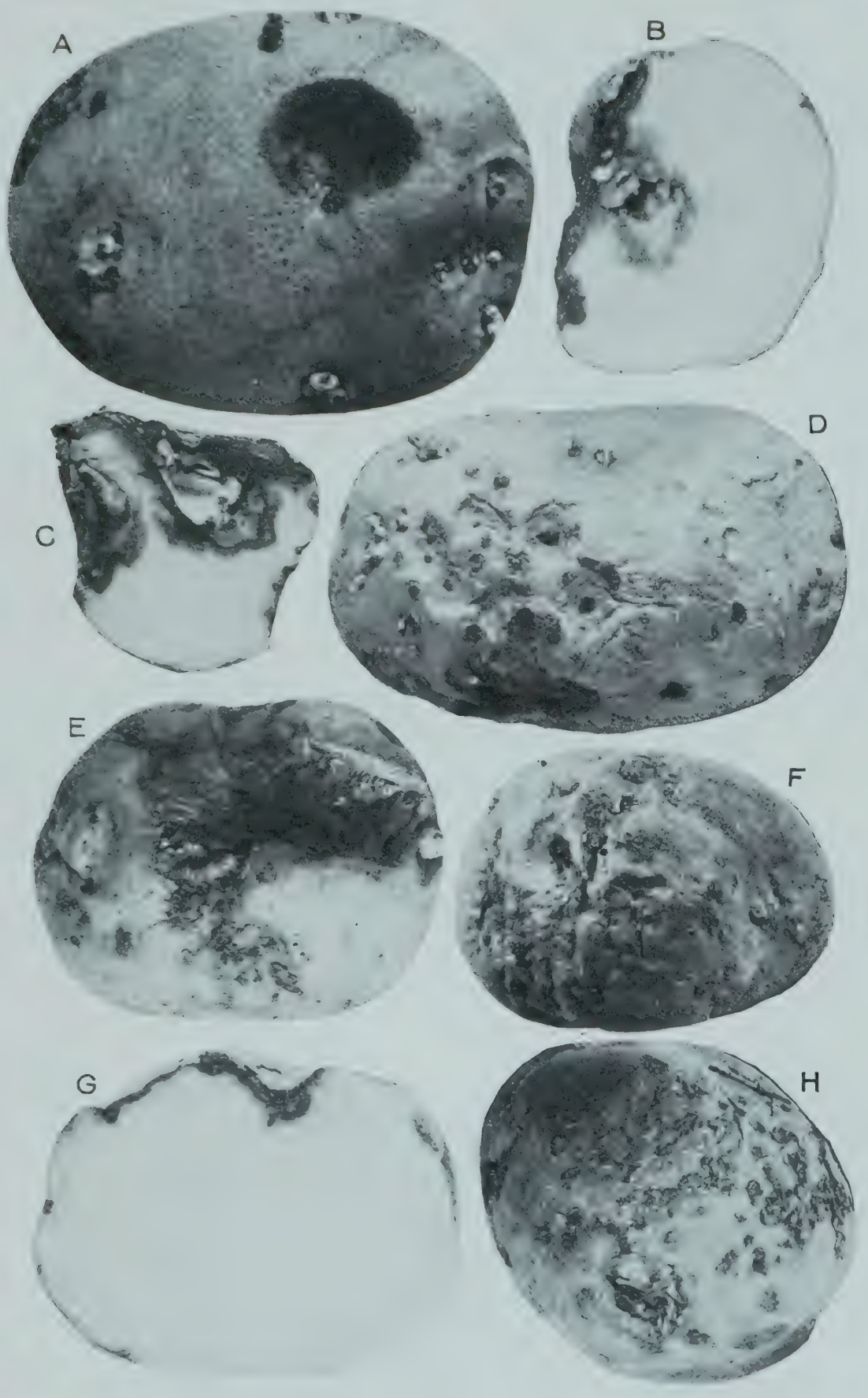
Fig. B, C.—Sections through tubers showing more advanced stages of a rot caused by a species of Phoma. A variety of other fungi have entered, causing further decay of the tuber.

Fig. D.—A potato tuber showing injury immediately around the sori, due partially to the work of the plasmodium. The lower side of the tuber also shows the beginning of the rot caused by *Phoma* sp.

Fig. E.—Infection due to *Phoma* sp. on a potato tuber infected with *Spongospora subterranea,* followed by another, due probably to *Fusarium coeruleum.*

Fig. F, H.—Potato tubers infected with *Spongospora subterranea* about three weeks after harvesting, showing the effects of desiccation injury.

Fig. G.—Section through a tuber, showing the depth to which rot caused by *Phoma* sp. extends. In this case no other fungi had entered.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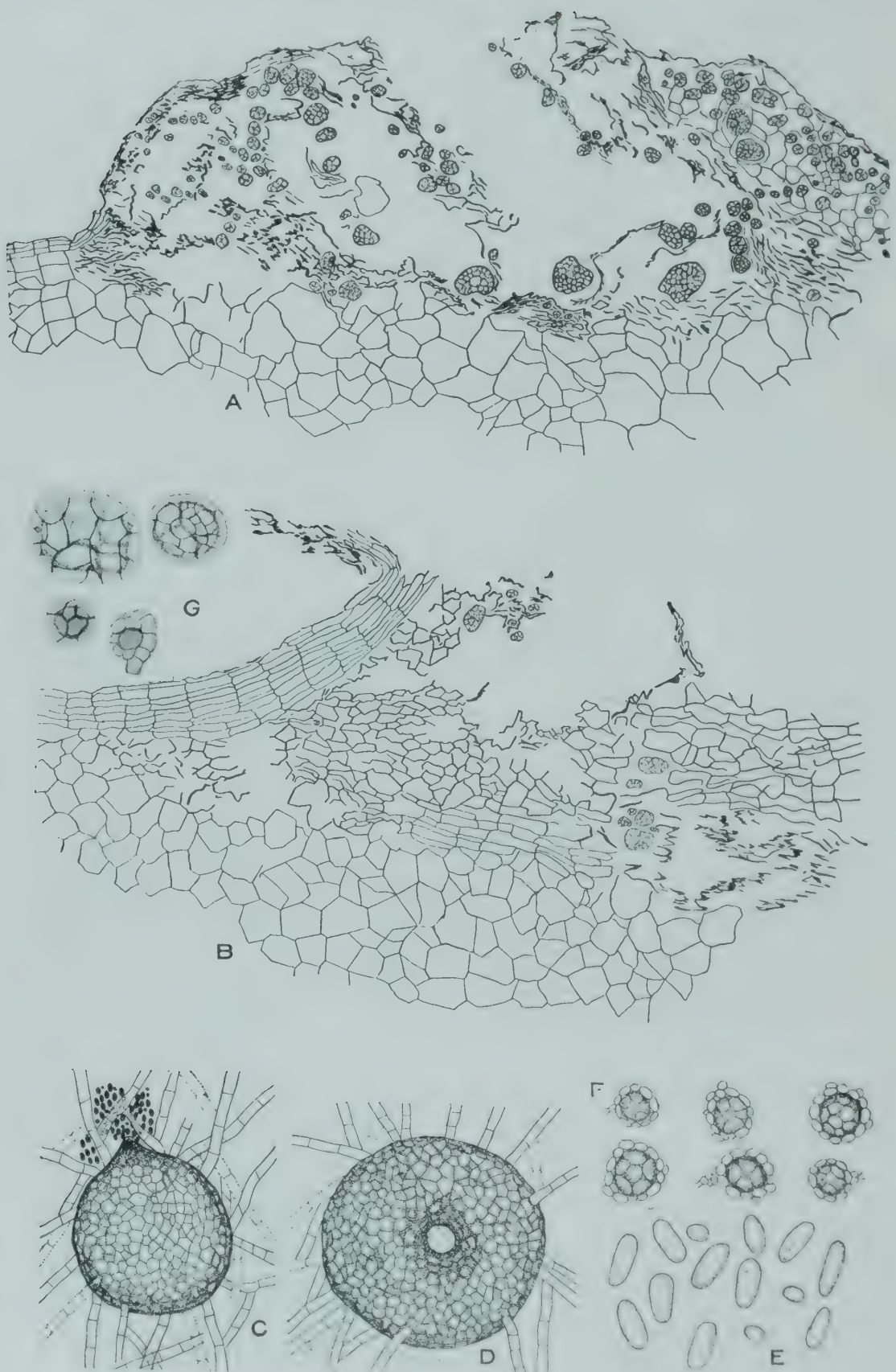
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PLATE 12

Spongospora subterranea and *Phoma tuberosa:*

Fig. A.—Section of a potato tuber through a sorus around which no dryrot has as yet set in. Spore balls are numerous and cork cells absent at the base of the sorus. ×110.

Fig. B.—Section of a potato tuber made through a sorus of *Spongospora subterranea* after the tuber had been held in storage and some dryrot due to desiccation had developed. A tendency toward the laying down of cork at the base is shown, but the cork cells have not yet thickened. ×110.

Fig. C, D.—Two views of the pycnidia of *Phoma tuberosa* as grown in pure culture. Figure C shows the pycnospores emerging from the ill-defined ostiole. ×650.

Fig. E.—Pycnospores. ×2,600.

Fig. F.—Mature "bulbils" of *Papulospora coprophila* (Zukal) Hotson, which in the tissues of potato tubers may be mistaken for spore balls of *Spongospora subterranea*. ×650.

Fig. G.—Spores of fungi associated with *Spongospora subterranea* and referred to *Verticillium* sp. and *Stysanus* sp. by Horne, of whose drawing this figure is a reproduction. Note the similarity to those shown in figure F.

PLATE 13

Phoma tuberosa on *Solanum tuberosum:*

Fig. A, B.—Stages of the rot caused by *Phoma tuberosa* on the Irish potato. The size and appearance of the lesion as shown in figure B is the most common. The abundance and size of the pycnidia vary.

Fig. C, D, E.—Results of artificial inoculation with pure cultures of *Phoma tuberosa*. Figure D shows an injured uninoculated tuber. Figure E shows the buttonhole lesion produced 10 days after inoculation. Figure C shows a tuber from another series three weeks after inoculation. Note the similarity of this lesion and that shown in Plate 12, figure A.

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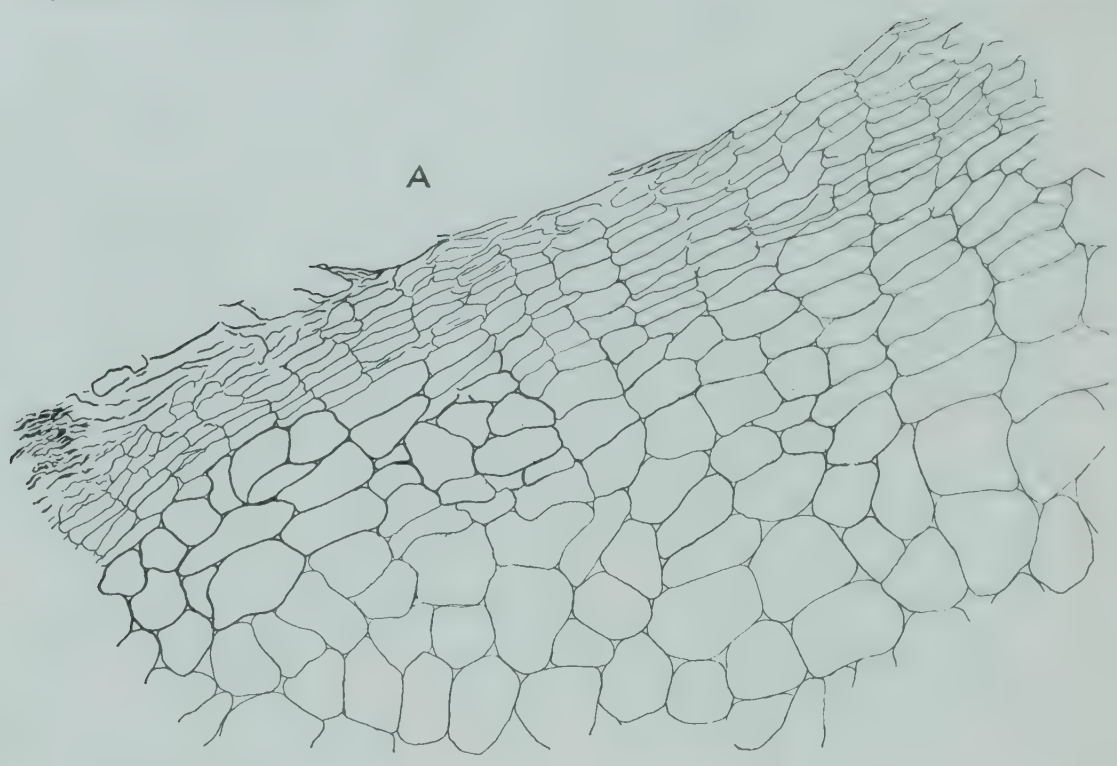
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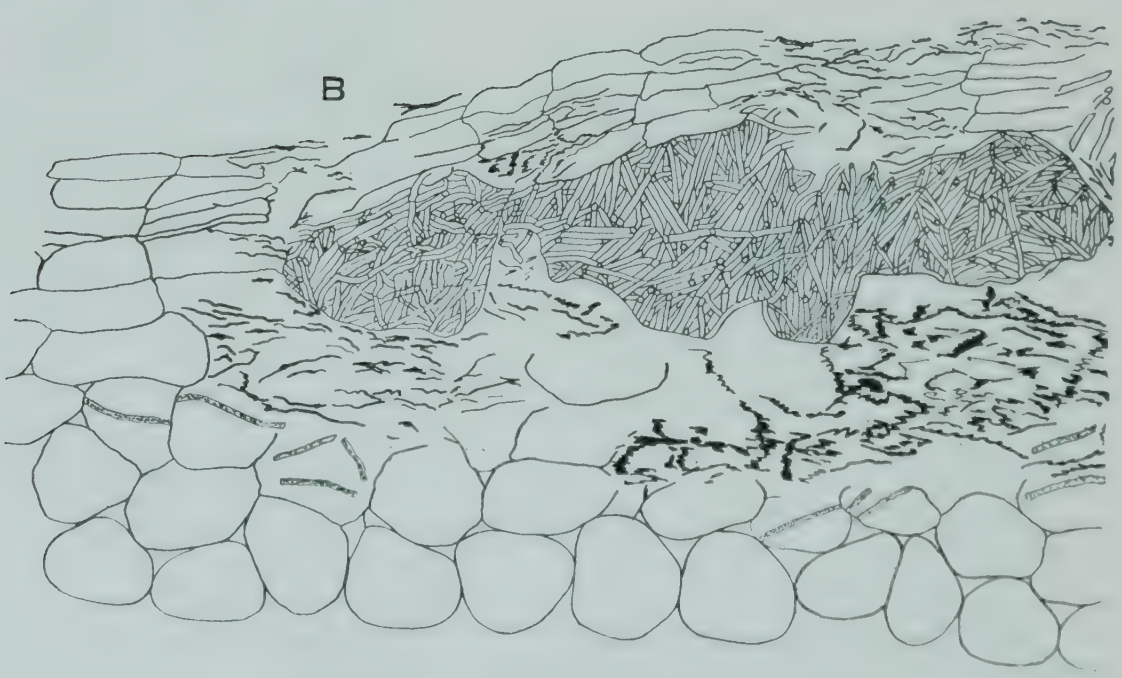
B

PLATE 14

Scab caused by *Phoma tuberosa* and *Oospora scabies* on *Solanum tuberosum:*

Fig. A.—Section through a tuber affected with common scab. The portion to the right is healthy tissue. Note the thickened cortex cells formed immediately below the epidermis. These cells prevent the desiccation of the tissues about the scab sorus. Generally such a condition is absent in tubers affected with scab caused by *Spongospora subterranea.* ×325.

Fig. B.—Section through a tuber affected with the rot caused by *Phoma tuberosa.* Note the mat of mycelium immediately below the epidermal cells. It is this mat that gives the lesion its hard, bony texture. The cells below the mat are broken down. It is in this cavity that other fungi begin their work. ×325.

GROWTH OF PARASITIC FUNGI IN CONCENTRATED SOLUTIONS

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The mycelium of a fungus growing parasitically is frequently in contact with the cell sap of its host plant. This cell sap is capable of an osmotic pressure which would vary, of course, with the amount and nature of the compounds in solution. The hyphæ of the invading parasite, then, may grow in a medium which has more or less high osmotic pressure. In order to grow in this medium, they must, of course, be able to withdraw water from it. It is apparent, then, that plant parasites must quite commonly possess the ability to withdraw water from more or less highly concentrated solutions and to grow in them.

The relative concentration of the cell sap of parasite and host has received some attention in the case of phanerogamic parasites. MacDougal (9, 10)[1] and MacDougal and Cannon (11) reached the conclusion that for their "Xeno parasites" the osmotic pressure of the parasite must be greater than the plant into which it is transplanted. Senn (14), in a recent investigation, has shown for certain phanerogamic parasites that the osmotic pressure of the parasite, as measured by the plasmolytic method, is invariably higher than that of its host. This writer seems not to have seen the work of MacDougal. Not so much attention has been paid to fungus parasites, though some work has been done in growing fungi in concentrated solutions. Eschenhagen (4) grew *Aspergillus niger*, *Penicillium glaucum*, and *Botrytis cinerea* on rather highly concentrated solutions of various substances. He found that these fungi grew in a saturated solution of potassium nitrate at ordinary temperatures and that the concentration of glucose which limited growth was above 50 per cent. He came to the conclusion that the ability of the fungi to live and grow when transferred to a higher concentration was due to a heightened osmotic pressure within the cell produced by an actual increase in the osmotically active substance therein.

Raciborski (13) also has shown that some fungi can live in exceedingly concentrated solutions. He grew *Torula* sp. in a saturated solution of lithium chlorid and *Aspergillus glaucus* in a similar solution of sodium chlorid. He considered the osmotic pressure in the cell to be greater than that of the outer medium and attempted to calculate the molecular

[1] Reference is made by number to "Literature cited," pp. 259–260.

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weight of a carbohydrate that would produce the necessary osmotic pressure. The present writer (7) germinated the conidia of *Glomerella cingulata* and grew the fungus in concentrations of calcium nitrate, potassium nitrate, and sucrose, which had diffusion tensions of 29.1, 39.3, and 47.3 atmospheres, respectively.

Dorn (3), in his study on the penetration of plant membranes by fungus hyphæ, mentions that there is an osmotic pressure of about 50 atmospheres in fungus hyphæ.

It seems probable from a consideration of the question and from the work that has been done that fungus parasites should be able to live and grow in solutions of a considerably higher concentration than the total concentration of the cell sap of their host plants. It was considered worth while, however, to obtain more evidence on this point. Ten common parasitic fungi were grown in solutions of salts and sugars of rather high concentrations. The total diffusion tensions of the dissolved materials in the expressed juice of some of their host plants was determined by the freezing-point method. The present paper deals with this work.

The fungi[1] studied were *Fusarium radicicola* Wollenw. and *F. oxysporum* Schlecht., two potato-rotting fungi (2); *Plenodomus destruens* Harter, *Diplodia tubericola* (E. and E.) Taub., *Sphaeronema fimbriatum* (E. and H.) Sacc., and *Rhizopus nigricans* Ehrenb., which are parasitic upon sweet potato (6), and *Botrytis cinerea* Pers., *Sclerotinia cinerea* (Bon.) Schröter, and *Sphaeropsis malorum* Peck, well-known parasites on the apple fruit. A strain of *Rhizopus nigricans* Ehrenb., which causes a serious rot of the strawberry (16), was also used. The data on the diffusion tension of the solutions in which *Glomerella cingulata* (Stonem.) S. and v. S. was grown are taken from an earlier paper by the present writer. Several of these fungi are parasitic on more than one of the hosts mentioned. The lowering of the freezing point of the expressed juice of apples (*Malus sylvestris*), sweet potatoes (*Ipomoea batatas*), potatoes (*Solanum tuberosum*), and strawberries (*Fragaria* spp.) was determined.

In the experiments with the fungi two methods were followed for the determination of the highest concentrations of the various substances used in which the fungi could grow. In the one method hanging-drop cultures were made of the spores which had been sown in salt or sugar solution of varying concentrations. These cultures were examined by means of a miscroscope, and the highest concentration in which the germination was apparent was noted. The other method was similar in principle to the one just outlined. In this the procedure was to sow the spores in sterilized tubes of the salt (sodium chlorid) or sugar solution and after about a week determine the growth or lack of growth by observation.

[1] The writer's thanks are due Mr. C. W. Carpenter for cultures of the species of Fusarium, Mr. L. L. Harter for cultures of the fungi from sweet potato, Dr. J. S. Cooley for the apple-rot fungi, and Dr. Neil E. Stevens for cultures of the species of Rhizopus from strawberry.

The spores were germinated and the fungi grown in solutions of calcium nitrate, potassium nitrate, sucrose, and glucose. Concentrated solutions were made of these substances, which were diluted down to the concentration desired in the experiments by the addition of distilled water to which a very little potato extract had been added.

The weight normal method of Morse and Frazer (12) was followed in making up the solutions.

The diffusion tension of the highest concentrations used in which the spores germinated and grew was calculated for the various compounds, taking into account, of course, the ionization of the salts. The data for calculating the percentage of ionization of the two salts were obtained from Jones's tables (8). The calculations of the percentage of ionization give probably only approximate values as the calculations are based on interpolations in most cases, and, moreover, some other substances were present which might influence the dissociation of the salts. The data thus obtained, however, probably offer a better basis for the comparison of the diffusion tensions of the two electrolytes and the two nonelectrolytes used in this study than the molecular concentrations given in the adjoining columns. The diffusion tension of the cane-sugar solutions was calculated from Morse and Frazer's determination of the osmotic pressure of a molecular solution of this substance at 25° C. From their work and from the determinations of Berkeley and Hartley (1) it seems quite probable that the values given are too low. The results obtained in growing fungi in the concentrated solutions of salts and sugars are given in Table I.

TABLE I.—*Highest concentrations (molecular) of calcium nitrate, potassium nitrate, sucrose, and glucose in which the fungi grew and the calculated diffusion tensions in atmospheres of these solutions*

Fungus.	Glucose.		Sucrose.		Potassium nitrate.		Calcium nitrate.	
	Concentration (molecular).	Diffusion tension (atmospheres).	Concentration (molecular).	Diffusion tension (atmospheres).	Concentration (molecular).	Diffusion tension (atmospheres).	Concentration (molecular).	Diffusion tension (atmospheres).
Fusarium radicicola	1.6	[a] 38.9	1.8	47.4	1.6	54.5	0.6	27.7
Fusarium oxysporum	1.6	[a] 38.9	1.8	47.4	1.6	54.5	.6	27.7
Plenodomus destruens	2.4	[a] 58.3	1.8	47.4	1.6	54.5	.7	33.6
Sphaeronema fimbriatum	2.6	[a] 63.2	1.8	47.4	1.6	54.5	.4	19.5
Diplodia tubericola	2.6	[a] 63.2	1.6	42.1	1.8	58.8	.7	33.6
Rhizopus nigricans (from strawberry)	1.6	[a] 63.2	.....	.....	.8	27.5	.3	15.9
Rhizopus nigricans (from sweet potato)	1.6	[a] 63.2	1.6	42.1	.8	27.5	.3	15.9
Botrytis cinerea	2.6	[a] 63.2	1.8	47.4	1.6	54.5	.6	27.7
Sclerotinia cinerea	.....	[a] 58.3	.....	.....	1.4	47.6	.6	27.7
Sphaeropsis malorum	2.6	[a] 63.2	1.8	47.4	1.6	54.5	.6	27.7
Glomerella cingulata	.....	.....	.....	41.3	.....	[a] 39.3	.....	29.1

[a] No higher concentrations used.

The freezing points of the expressed juice of apples, sweet potatoes, potatoes, and strawberries were determined by means of a Beckman freezing-point apparatus, and the total diffusion tension for the material in these solutions was calculated from the lowering of the freezing point (15).

The results obtained by the calculation of these data are given in Table II.

TABLE II.—*Diffusion tension in atmospheres of the juice of certain hosts of the fungi studied, as calculated from the lowering of the freezing point*

Host.	Diffusion tension (atmospheres.)
Strawberry	8.27
Apple (Blacktwig)	17.85
Sweet potato (Jersey Big Stem)	10.25
Potato (Irish Cobbler)	6.52

From Table I it is evident that the fungi used in these experiments are able to grow in relatively high concentrations of salts and sugars. The highest diffusion tension of any solution in which growth was evident was in the concentrated solutions of glucose. Growth occurred in all concentrations of this sugar used.

With potassium nitrate growth was inhibited in all cases when the diffusion tension of the solution was about 59 atmospheres; and in only one case was a fungus able to grow in a solution so concentrated. All the fungi except the two strains of *Rhizopus nigricans* and *Glomerella cingulata* grew in concentrations which have a calculated diffusion tension of 47 atmospheres.

The fungi also grew in solutions of sucrose of rather high concentrations, the two strains of *Rhizopus nigricans* and *Diplodia tubericola* being the only fungi which were unable to grow in sucrose solutions having a diffusion tension of 40 atmospheres. It is noticeable that growth in the case of *R. nigricans* is inhibited also at lower concentrations of potassium and calcium nitrates than with the other fungi.

Calcium nitrate inhibited germination and growth always at concentrations considerably lower than those required to produce the same effect with sucrose, glucose, and potassium nitrate. Nevertheless a comparison of the diffusion tensions of the highest concentrations of calcium-nitrate solutions in which the fungi grew and the diffusion tension of the juice of the host plant as calculated from the lowering of the freezing points shows that the parasite is in all cases able to grow in considerably higher concentrations than are present in the cell sap of its host plant. Whether or not the protoplasm of the fungi used is impermeable to the two salts and two sugars used in these experiments

is a question which can not be answered from the data now at hand. Potassium nitrate has been used extensively as a plasmolyzing agent for determining osmotic pressure in plants.

Fischer (5) reaches the conclusion, however, that the protoplasts of bacteria are readily permeable to this salt. True (17) used cane sugar in his work on species of Spirogyra, and it has been used to a considerable extent by other investigators. Calcium nitrate is not commonly used as a plasmolyzing agent.

From the concentrations of glucose which were found to be favorable to the growth of fungi it seems probable that this substance penetrated the protoplasm, perhaps quite readily. It is, of course, probable that all the substances penetrate into the cell to some extent. It is possible that the protoplasm of the fungi here used is readily permeable to the salts and sugars employed in this study and that they can pass into the cell until the concentration within is the same as without. Then the presence within the cell of some substance to which the protoplasm was impermeable would raise the osmotic pressure within above that of the surrounding solution, and the fungus could grow.

The importance of this ability of parasitic fungi to grow in solutions which are capable of exerting a high osmotic pressure is evident. Whether this ability is due to the osmotic pressure in the fungus being originally higher or whether it becomes higher through a diffusion of substance into the hyphæ, or whether there is an actual increase in the osmotically active substances within the cell, as Eschenhagen concludes, or whether other factors than a high osmotic pressure enable fungi to remain turgid and grow in concentrated solutions are questions which need further investigation.

In these experiments in which fungi were grown in solutions of potassium and calcium nitrate, sucrose, and glucose it was found that in every case the fungi grew readily in solutions in which the diffusion tensions were much higher than the total diffusion tensions of the dissolved substances in the juices of their host plants.

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FREEZING-POINT LOWERING OF THE LEAF SAP OF THE HORTICULTURAL TYPES OF PERSEA AMERICANA

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INTRODUCTION

In the introduction of tropical economic plants into the warmer portions of the United States, which for the most part are not free from at least occasional frosts, ability to survive transient low temperature is a characteristic of such fundamental importance that any quantitative contribution to our knowledge of its physiology must be of value. Among the factors to which frost resistance in plants is due, the magnitude of the depression of the freezing point of the cell sap has been suggested as one of importance.

That the freezing-point lowering of the cell sap is not in itself a sufficient explanation of differences in frost resistances should be quite obvious from the narrowness of its range in plants of economic importance. To what degree the osmotic pressure of the sap may be correlated with other characteristics which are of significance in cold resistance remains to be investigated.

A discussion of the several possible factors or references to the very extensive literature of the subject fall quite outside the scope of a note which is designed only to present a series of constants pertinent to the problem of hardiness in a particular tropical fruit.

The Plant Introduction Field Station at Miami, Fla., has seemed to the writers to afford particularly advantageous materials for a test of the existence of a relationship between capacity for cold resistance and the freezing-point lowering of the extracted sap, since it contains, assembled under the same environmental conditions, a wider range of varieties than can be found elsewhere.

The avocado, *Persea americana* Miller (*Persea gratissima* Gaertn. f.), while introduced to Florida and California a good many years ago, has only been propagated asexually since the beginning of the present century. Hence, the number of horticultural varieties is not overwhelmingly large. Some of these varieties have been under observation

by horticulturists for a period of 5 to 15 years, and records as to comparative hardiness have been obtained. These records show that the horticultural types differ significantly in regard to the amount of frost to which they can be subjected without injury.

The cultivated avocados fall naturally into three groups, or types, each of which possesses distinguishing characteristics which are quite constant, though occasional forms are seen which appear to be intermediate or to belong to distinct groups not yet well known in cultivation. The characteristics of the three cultivated types are briefly as follows:

MEXICAN TYPE.—Foliage and sometimes the fruit distinctly anise-scented. Leaves usually smaller than those of the Guatemalan and West Indian types. Fruit commonly 4 to 8 ounces in weight; skin thin, often membranous, usually smooth and glossy on the surface. Seed coats thin; closely united and adhering to the cotyledons, or separating, as in the West Indian type. Flowers heavily pubescent, appearing in early spring, from January to March in California and Florida. Ripe fruits from June to October. Occasionally a second crop, from later bloom, ripens in winter and spring.

GUATEMALAN TYPE.—Foliage not anise-scented, deep green, the new growth bronze-red, commonly, but not always, deeper colored than that of the West Indian type. Fruits commonly from 12 to 18 ounces in weight; skin usually verrucose or tuberculate on the surface, one-sixteenth to three-sixteenths of an inch thick, woody, brittle, and coarsely granular in texture, sharply differentiated from the flesh. Seed, as a rule, comparatively small; cotyledons smooth; the seed coats thin, closely united, and adhering to the cotyledons throughout. Flowers more finely pubescent than in the Mexican type, appearing in late spring—March to May in Florida. Fruits matured in the winter or spring of the following year.[1]

WEST INDIAN TYPE.—Foliage not anise-scented; generally similar to the Guatemalan, but the young branchlets and leaves often lighter in color. Fruits variable in form and size, as in the other types, comparatively large, averaging 14 to 20 ounces, but sometimes weighing 3 pounds or more; surface nearly always smooth, the skin rarely more than one-sixteenth of an inch thick, pliable and leathery, and scarcely so well differentiated from the flesh as in the Guatemalan. Seed usually large in proportion to the size of the fruit; cotyledons more or less rough; the two seed coats frequently thick and separated, at least over the distal end of the seed, one adhering to the cotyledons and the other loose or adhering to the wall of the seed cavity. Flowers usually as pubescent as those of the Guatemalan type, occasionally glabrate; in Florida and the West Indies they appear from February to April. Matured fruits from July to November.

[1] Some of the varieties of this type have the skin nearly smooth and about the same thickness as that of the West Indian. For this reason they have been considered by a few horticulturists as forming a distinct class; but inasmuch as they seem to differ only in these two points, which are both variable characters at best, it seems safe to retain them in the Guatemalan type.

EXPERIMENTAL METHODS

The technique employed in determining freezing-point lowering was very simple. Fully matured but still normally green leaves were collected and frozen in an ice and salt mixture to facilitate the extraction of sap by pressing [1] in a small heavily tinned bowl under a powerful screw. The freezing-point lowering was determined by means of a mercury thermometer graduated to 0.01 of a degree centigrade in subdivisions sufficiently large to allow of fairly accurate estimation to smaller fractions. The vaporization of ether or carbon bisulphid in a vacuum jacket was used in determining the freezing-point lowering. The results are expressed in terms of freezing-point lowering in degrees centigrade (Δ), corrected for undercooling by the usual formula.

For those who prefer to think in terms of osmotic pressure the values of P are given from a published table.[2] Finally, for the convenience of those who wish to know the actual freezing point of the saps on the Fahrenheit scale, these values have been added (F).

PRESENTATION OF CONSTANTS FOR THE THREE TYPES[3]

MEXICAN TYPE.—Throughout the highlands of central and northern Mexico this type is very common. Because of its superior hardiness, it has been extensively planted in several subtropical regions, most notably in California and Chile. It is known to have been planted in California as early as 1870. In Florida it has fruited as far north as Gainesville, but is not generally cultivated in any part of the State. It has been planted along the Riviera in southern Europe and has fruited in the open at Rome. In Algeria it has also been planted, though to a very limited extent. In the West Indies it is scarcely known.

During the cold weather of January, 1913, in California trees of this type were reported to have withstood temperatures of 16° to 20° F. without injury.

1. Querétaro..... $\Delta=1.11$, $P=13.3$, $F=30.00°$
Buds received from California, where the variety was introduced from Querétaro, Mexico, in 1911. Cion 1 year old, on one branch of No. 4, Mexican stock.

2. San Sebastian..... $\Delta=1.20$, $P=14.5$, $F=29.84°$
Buds received from California, where the variety was introduced from Querétaro, Mexico in 1911. One-year-old cion on Mexican stock, a branch of No. 4.

3. Harman..... $\Delta=1.24$, $P=15.0$, $F=29.77°$
Originated at Sherman, Cal., budded on a limb of 26713, West Indian type. Cion 2 years old.

4. Seedling..... $\Delta=1.27$, $P=15.2$, $F=29.71°$
Grown from seed of unknown origin.

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[1] Gortner, R. A., and Harris, J. A. Notes on the technique of the determination of the depression of the freezing point of vegetable saps. *In* Plant World, v. 17, No. 2, p. 49–53, 1914.

[2] Harris, J. A., and Gortner, R. A. Notes on the calculation of the osmotic pressure of expressed vegetable saps from the depression of the freezing point, with a table for the values of P for $\Delta=0.001°$ to $\Delta=2.999°$. *In* Amer. Jour. Bot., v. 1, no. 2, p. 75–78. 1914.

[3] The numbers following the varietal names refer to the Inventory of the Office of Foreign Seed and Plant Introduction, Bureau of Plant Industry.

5. Harman.....Δ=1.29, *P*=15.6, *F*=29.68°
Originated at Sherman, Cal. Two-year-old cion on one branch of No. 4, Mexican stock.

6. Seedling (19206).....Δ=1.30, *P*=15.7, *F*=29.66°
Grown from seed received from Coahuila, Mexico.

7. Seedling.....Δ=1.30, *P*=15.6, *F*=29.66°
Grown from seed of unknown origin.

8. Seedling.....Δ=1.30, *P*=15.6, *F*=29.66°
Grown from seed of unknown origin.

9. Seedling.....Δ=1.36, *P*=16.3, *F*=29.55°
Grown from seed of unknown origin.

10. Seedling (34831).....Δ=1.37, *P*=16.5, *F*=29.53°
Budded tree from a seedling growing in Rome, Italy.

11. Seedling (32400).....Δ=1.39, *P*=16.7, *F*=29.50°
Originated at Orange, Cal. Budded tree.

12. Fuerte.....Δ=1.41, *P*=16.9, *F*=29.46°
Buds received from California, where the variety was introduced in 1911 from State of Puebla, Mexico. One-year-old cion on branch of No. 33, West Indian stock.

13. Seedling.....Δ=1.43, *P*=17.2, *F*=29.43°
Grown from seed of unknown origin.

GUATEMALAN TYPE.—This group was first called to the attention of horticulturists by Collins in 1905.[1] Within the last few years it has been extensively planted in California and is now becoming known in Florida. It is found commonly in the mountainous parts of Guatemala and northward into southern Mexico, whence have come many of the cultivated varieties now being propagated in the United States. It was introduced to Hawaii about 20 years ago, according to Higgins, Hunn, and Holt,[2] while it appears to have been first planted in California about 1885. In Florida it was probably not introduced earlier than 1900. It has not been observed in the West Indies, with the exception of a few trees recently planted, and its distribution in other countries is quite limited.

In California, where it is best known horticulturally, it has been found considerably hardier than the West Indian type, but somewhat more tender, as a rule, than the Mexican.

14. Seedling (10978).....Δ=1.09, *P*=13.1, *F*=30.04°
Grown from seed introduced from Guatemala in 1904.

15. Colorado.....Δ=1.16, *P*=13.9, *F*=29.91°
Budded on one limb of No. 24, West Indian type. Originated at Los Angeles, Cal., the seed having been sent from the State of Puebla, Mexico. Cion 1 year old.

16. Taft.....Δ=1.25, *P*=15.0, *F*=29.75°
Originated at Orange, Cal. Two-year-old cion on limb of No. 4, Mexican stock.

17. Seedling (38549).....Δ=1.34, *P*=16.2, *F*=29.59°
Originated at Antigua, Guatemala. Budded on one limb of (26694), West Indian type.

18. Sinaloa.....Δ=1.35, *P*=16.2, *F*=29.57°
Buds obtained from California, where the variety was introduced in 1911 from the State of Puebla, Mexico. Cion 1 year old, on limb of No. 27, West Indian type.

[1] Collins, G. N. The avocado, a salad fruit from the Tropics. U.-S. Dept. Agr. Bur. Plant Indus. Bul. 77, 52 p., 8 pl. 1905.

[2] Higgins, J. E., Hunn, C. J., and Holt, V. S. The avocado in Hawaii. Hawaii Agr. Exp. Sta. Bul. 25, 48 p., illus., 7 pl. 1911.

19. Nutmeg.....Δ=1.38, *P*=16.5, *F*=29.52°
Buds obtained from Hawaii, where the variety originated from a seed received from Guatemala. Two-year-old cion on West Indian stock.

20. Seedling (36603).....Δ=1.38, *P*=16.6, *F*=29.52°
Budded tree. Original grown at Honolulu, Hawaii, from a seed imported from Guatemala.

21. Seedling (19080).....Δ=1.39, *P*=16.8, *F*=29.50°
Tree introduced as a plant from Guatemala in 1906.

22. Seedling (19058).....Δ=1.40, *P*=16.8, *F*=29.48°
Grown from seed introduced from Guatemala in 1906.

WEST INDIAN TYPE.—This is the commonest type on tropical American seacoasts. Its precise origin is not known, but since it has been demonstrated by Collins[1] that the avocado was not cultivated in the West Indies in pre-Columbian times it must have been introduced to the islands from some point on the mainland. At the present day it is practically the only type grown in the West Indies, and it is known to be common in Colombia, Venezuela, Brazil, and Peru. It occurs in the Mexican lowlands and in Yucatan. Most of the avocados grown in Hawaii belong to this type, as do those of Tahiti, some of which are occasionally shipped to San Francisco. In south Florida it is the principal type cultivated, having been introduced probably from Cuba at an early day and planted on both the east and west coasts. In California its cultivation is very limited. It is found in the Canary Islands and has been introduced to India, but in this latter country avocado culture is of no importance.

Horticulturally the most important feature of this type is its susceptibility to cold, which prevents its culture in any but the warmest sections of the United States. Experience has shown it to be severely injured by a temperature at least 4 or 5 degrees Fahrenheit higher than that required to injure the Guatemalan type.

23. Seedling (26707).....Δ=0.96, *P*=11.6, *F*=30.27°
Originated at Fulford, Fla. Budded tree.

24. Seedling (26692).....Δ=1.00, *P*=12.0, *F*=30.20°
Originated at Santiago de las Vegas, Cuba. Budded tree.

25. Seedling (26693).....Δ=1.03, *P*=12.4, *F*=30.15°
Originated at Cocoanut Grove, Fla. Budded tree.

26. Seedling (26704).....Δ=1.03, *P*=12.4, *F*=30.15°
Originated at Miami, Fla. Budded tree.

27. Largo (18730).....Δ=1.04, *P*=12.5, *F*=30.13°
Originated at Nassau, Bahama Islands. Budded tree.

28. Seedling (26698).....Δ=1.04, *P*=12.5, *F*=30.13°
Originated at Fort Myers, Fla. Budded tree.

29. Seedling (19297).....Δ=1.06, *P*=12.8, *F*=30.09°
Originated at Cocoanut Grove, Fla. Budded tree.

30. Seedling (36270).....Δ=1.07, *P*=12.9, *F*=30.07°
Grown from seed of unknown origin.

31. Seedling (26703).....Δ=1.08, *P*=12.9, *F*=30.06°
Originated at Buena Vista, Fla. Budded tree.

[1] Collins, G. N. Op. cit.

32. Seedling (26694).....Δ=1.09, P=13.2, F=30.04°
Originated at Marco, Fla. Budded tree.

33. Seedling.....Δ=1.09, P=13.1, F=30.04°
Grown from seed of unknown origin.

34. Butler (26690).....Δ=1.10, P=13.3, F=30.02°
Originated at St. Petersburg, Fla. Budded tree.

35. Seedling (26691).....Δ=1.15, P=13.9, F=29.93°
Originated at Buena Vista, Fla. Budded tree.

36. Mitchell (18120).....Δ=1.19, P=14.3, F=29.86°
Originated at Bayamon, Porto Rico. Budded tree.

37. Seedling (26713).....Δ=1.23, P=14.8, F=29.79°
Originated at Cocoanut Grove, Fla. Budded tree.

38. Seedling (19379).....Δ=1.23, P=14.8, F=29.79°
Grown from a seed received from Hawaii.

39. Pollock.....Δ=1.24, P=14.9, F=29.77°
Originated at Miami, Fla. Budded tree.

DISCUSSION OF CONSTANTS

All these types show considerable variation in freezing-point lowering. For the whole series the range is from Δ=0.96° to Δ=1.43°, or 0.47°. On the Fahrenheit scale the range in the freezing point of the sap is from 29.43° to 30.27°. This variation is doubtless due to many causes. In addition to errors of sampling in the collection of the leaves from the individual trees and the unavoidable errors of measurement involved in the manipulation of the sap samples, there are the fluctuations attributable to the uncontrollable differences in the physiological states of the different trees at the time of collecting the materials.

In view of these various factors, one would not expect to find transition types entirely wanting, unless the differentiation of the types be very clearly marked indeed. For more convenient comparison the values obtained from the trees of the three types may be serially arranged in intervals of five-hundredths of a degree (Table I).

TABLE I.—*Comparison of the freezing-point lowering values of three types of Persea americana*

Depression of freezing point.	Guatemalan type.	Mexican type.	Guatemalan and Mexican types.	West Indian type.
° C.				
0.91–0.95	.....	.....	.....	.....
0.96–1.00	.....	.....	.....	2
1.01–1.05	.....	.....	.....	4
1.06–1.10	1	.....	1	6
1.11–1.15	.....	1	1	1
1.16–1.20	1	1	2	1
1.21–1.25	1	1	2	3
1.26–1.30	.....	5	5	.....
1.31–1.35	2	.....	2	.....
1.36–1.40	4	3	7	.....
1.41–1.45	.....	2	2	.....
1.46–1.50	.....	.....	.....	.....
	9	13	22	17

An inspection of the values in Table I shows no clear difference between the plants of the Guatemalan and Mexican types. From both of these the West Indian type seems to be differentiated by a distinctly slighter freezing-point lowering. All but 3 of the 17 West Indian determinations are lower than 1.20°, whereas all but 4 of the 22 based on plants of the Guatemalan and Mexican types show a freezing-point lowering of 1.21° or more.

If the averages of the freezing-point lowerings (Δ) with their probable errors be determined as a more exact means of comparison from the ungrouped depressions given for the individual varieties above, the following constants are obtained (Table II).

TABLE II.—*Freezing-point lowering constants of three types of Persea americana*

Type.	Mean.	Standard deviation.	Coefficient of variation.
Guatemalan	1.304±0.024	0.106±0.017	8.16
Mexican	1.305± .016	.087± .012	6.68
Guatemalan and Mexican	1.305± .014	.095± .014	7.25
West Indian	1.096± .014	.083± .010	7.55

The average freezing-point lowering in the Guatemalan and Mexican types is practically the same. The difference is only 0.001 ±0.029 of a degree. The West Indian type is characterized by a distinctly lower average than either of the other types. The differences are:

West Indian and Guatemalan type..... −0.209±0.027°
West Indian and Mexican type..... −0.210±0.021°
West Indian and Guatemalan and Mexican types..... −0.209±0.019°

These differences are 7.6, 9.9, and 11 times as large as their probable errors. Thus, notwithstanding the relatively small number of observations upon which this study has of necessity been based, the differences seem to be quite trustworthy in comparison with their probable errors.

Within the type the absolute variation in freezing-point lowering is very slight, amounting to one-tenth of 1 degree or less. The relative variability as expressed in terms of the coefficient of variation is also low for a plant character.

SUMMARY

The constants presented in this paper prove that in a tropical fruit of relatively recent introduction to North American horticulture, the avocado, one of the groups of varieties, the so-called West Indian type, is characterized by tissue fluids which freeze at a distinctly higher temperature than in the two other groups of varieties (Guatemalan and Mexican). In the conventional terms of physical chemistry adopted by physiologists, the expressed leaf sap of West Indian type varieties is characterized by a slighter depression of the freezing point or by a slighter freezing-point lowering than is that of the two other groups of

varieties. This differentiation seems to hold with remarkable constancy notwithstanding the wide geographic origin—West Indian, Bahaman, Central American, Mexican, and Hawaiian—of the seeds or budwood from which the tissues dealt with originated.

The type which is characterized by the slightest freezing-point lowering of its extracted sap—that is, the type in which the expressed sap freezes at the highest temperature—is the one which has been shown by horticultural experience to be the least capable of enduring cold. That capacity to withstand low temperatures is not solely due to differences in the freezing point of the sap is evident from the slightness of the differences in the cryoscopic constants of the West Indian as compared with the Mexican and Guatemalan types. Furthermore, horticulturists believe that the plants of the Guatemalan type are intermediate in hardiness between those of the Mexican and West Indian types. There is, so far as our data go, no discernible difference in the freezing point of the sap of these types.

The problem is evidently one of considerable complexity. To what extent other characteristics contributing to the capacity of the organism to withstand low temperatures are correlated with sap properties remains to be investigated.

It seems highly probable from the evidences presented in the paper that in the case of the tropical perennials, a knowledge of the freezing-point lowering of the sap would be of some service in predicting ability to withstand cold. At least the subject is one deserving of more extensive investigation. We would have been glad to carry out the present study on a far more extensive scale, but the determinations given practically exhaust the trees of flowering age in the collection of Guatemalan and Mexican types at the Miami Plant Introduction Field Station, and it will probably be several years before a better series is available.

GRAIN OF THE TOBACCO LEAF

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INTRODUCTION

When the buyer examines a sample of cigar leaf, he takes into account such factors as texture, elasticity, color, luster, thickness, and grain. He rolls a trial cigar and considers the burn, flavor, aroma, and character of the ash. These he judges through long experience and by comparison with an arbitrary standard for the crop of that particular year and the type of leaf in determining the price he will pay for the tobacco of which the sample is representative. Though the character of the grain itself, whether it be fine or coarse, close or open, may be a relatively minor consideration in fixing the value of a given sample, it is undoubtedly closely correlated with some of the other factors considered and is therefore of more importance than has hitherto been supposed.

OCCURRENCE AND GENERAL MACROSCOPIC APPEARANCE OF THE GRAIN

The usual form of the grain of tobacco appears to the unaided eye as minute pimples or papillæ slightly raised above the general surface of the leaf tissue. They are more prominent on the upper surface and vary in size from about 1 mm. in diameter down to bodies so small as to be entirely invisible without the aid of the microscope. When a well-cased leaf is stretched over the ball of the finger, the grain becomes more conspicuous (Pl. 15, fig. A and B), and, indeed, it is in this way that the tobacco buyer or grader determines the size and distribution of these bodies. If a leaf with a coarse grain be pressed between the thumb and finger, it feels as though particles of sand were adhering to it. Some of the larger grain bodies can be picked out with the point of a knife; and when such a grain is pressed between two hard substances, it crushes in the same manner as would a particle of crystal, for the grain itself is a crystalline body. On burning, these grain bodies swell and cause the pearl-like pimples so frequently seen on the ashes of cigars (Pl. 15, fig. C and D). Grain is a characteristic of cigar tobacco, both domestic and imported (7).[2] In the flue-cured types macroscopically visible crystalline deposits seem to be entirely absent. However, it is reasonable to suppose that all air-cured types possess grain in some degree.

[1] The writer wishes to express his gratitude for helpful suggestions and kind criticisms made by Dr. W. W. Garner, for inorganic and organic analyses made by Mr. C. L. Foubert and Dr. C. W. Bacon, respectively, and for kind cooperation on the part of Mr. Otto Olson in the matter of obtaining material.

[2] Reference is made by number to "Literature cited," p. 273.

With two notable exceptions the grain particles are entirely embedded in the tissue of the leaf, being distributed more or less evenly in the web and along the veins. One of the superficial types appears as raised black dots, which are found chiefly on dark-colored leaves of a heavy texture. The other is composed of those which give somewhat the appearance of minute disks situated on or immediately beneath the surface on either side of the leaf. These latter seem most conspicuous on leaves of a medium or light color.

MICROSCOPIC CHARACTERS OF THE GRAIN

When a cased leaf of tobacco containing grain is stretched over the stage of the microscope and examined in ordinary transmitted light with a low magnification, most of the larger grain particles appear as more or less rounded, highly refractive bodies of compact structure and bright reddish brown color. Indeed, the ground tissue appears uniformly light in color in comparison with that of the leaf observed macroscopically, and it is seen that by far the larger part of the brown color of the leaf is localized in the grain and in some of the small veins. Even when one looks with a hand lens through a moist leaf against a strong source of light, this localization of color in the grain is evident; the effect is that of minute garnets embedded in a yellowish brown matrix.

In material mounted in Canada balsam, after having been dehydrated in alcohol and cleared in xylol, the grain bodies become less conspicuous in ordinary transmitted light, inasmuch as their refractive index more nearly approaches that of the balsam than that of the air. The bodies may be located, however, because of their difference in color from the surrounding ground tissue, owing to the concentration in them of the brown coloring matter and their slight power of refraction of light in balsam (Pl. 16, fig. A).

It is in polarized light that the grain bodies become most conspicuous, since, as stated before, they are of crystalline structure. Favorable material mounted in balsam then exhibits clearly their size, shape, and structure, as well as showing the degree to which the grain material has been brought together into definite aggregates, a point which will be mentioned later in this paper.

VARIOUS FORMS OF GRAIN

There are five general types of grain which merge insensibly into one another and which may be briefly described in their order of abundance, as follows: The first and most common type consists of more or less spherical masses each composed chiefly of a group of palisade cells distended with minute, radiating, needle-shaped crystals of a brown color (Pl. 16, fig. B). The second type includes flat or roughly hemispherical bodies composed of cells of the mesophyll and epidermis, which are filled with light brown to almost colorless, comparatively large plates arranged somewhat regularly (Pl. 16, fig. C). The third is similar to the

first type, in that the bodies are usually spherical; but here the surface is decidedly nodular and the cells included may be either the palisade or the spongy parenchyma, or both. The crystals are radially arranged in small groups the individuals of which appear to be thin, narrow plates, and the color is more gray than brown (Pl. 16, fig. D).

The fourth type attains the largest size and always has one surface in common with the surface of the leaf. The particles consist of a number of cells, palisade and epidermal, filled with a mass of dark-brown or black substance which in the unbroken particle is inactive in polarized light. When crushed, however, the fragments between crossed Nicol prisms show the presence of crystalline material the form of which is not apparent. These dark bodies are very striking, in that the epidermal surface is usually craterlike in appearance, having a concavity which frequently contains a few minute, colorless crystals surrounded by a raised black ring. In a few of this type the central portion of the upper surface shows no concavity, but radiating lines extending from a centrally located spot beneath which the substance of the particle is soft and easily crumbled suggest that this spot marks the location of the base of one of the large trichomes or, possibly, the position of a stoma which failed to close during the curing process (Pl. 16, fig. E).

The fifth type of grain is composed entirely of microscopic sphere crystals which are very active in polarized light and plainly show the four extinction bars which rotate upon revolving the analyzer. Unlike the other types, they usually do not fill the cells in which they occur. They are colorless or light brown in ordinary transmitted light and possess no visible differentiation into individual crystals (Pl. 16, fig. F). Tunmann (8, p. 147) indicates that these spherites, which he believes to be malic-acid salts, separate out when dried tobacco is placed in alcohol. The writer has observed that they are formed in the leaf during the process of curing and that in some instances they are visible under the microscope in the untreated, dry leaf with the aid of strong polarized light, even after fermentation has been completed.

OTHER CRYSTALLINE MATERIAL OF THE LEAF

Aside from the grain, two other types of crystalline material are found in the tissue of the cured and fermented tobacco leaf. One of these is the cryptocrystalline or sand crystals of calcium oxalate contained in certain cells (idioblasts) which are always present in all tobacco, even in the green leaf while it is still attached to the growing plant, and which show no appreciable change during the process of curing or fermentation. The other is that which appears in nearly every cell of the leaf in both green and cured tobacco and consists of small, single prisms. Some of the properties of both of these types will be mentioned in connection with a consideration of some of the chemical characteristics of the grain (Pl. 16, fig. A, and Pl. 17, fig. A).

METHOD OF SEPARATION OF GRAIN FROM THE LEAF AND MECHANICAL ANALYSIS OF THE LEAF

Preliminary investigations indicated that a considerable supply of the grain bodies free from the surrounding tissues of the leaf would be desirable. A method of mechanical separation was therefore worked out, which, in brief, is as follows: A quantity of air-dry cigar leaf tobacco was rubbed through a series of sieves (from 10 to 150 mesh) with a flat pestle made from a rubber stopper. This process removed a large amount of the soft web of the leaf from the veins as well as from the hard grain particles, at the same time breaking the veins into short lengths, but not crushing the grain. The mixture of grain and vein remaining in each sieve was then ground in an unglazed porcelain mortar with a rounded rubber pestle. This served to remove any soft tissue still adhering to the particles of grain and vein. After sifting out with the appropriate sieve the small amount of fine material thus rubbed off, the mixture of grain and vein remaining was slowly poured upon a piece of smooth paper inclined at an appropriate angle, determined by experiment. The more or less spherical particles of grain rolled down the inclined paper more rapidly than the cylindrically-shaped pieces of vein, resulting in a partial separation of the former from the latter. It was found, however, that a large amount of the grain belonging to the flatter types remained with the vein, and it was therefore necessary to pick this grain out of the mixture with the aid of a binocular microscope. By these tedious means about 30 or 40 gm. of practically pure grain were obtained for subsequent detailed study.

The separation of the grain and vein from the soft tissues of the leaf in this manner in one instance also resulted in an approximate mechanical analysis of the tobacco leaf. It was found that of a 70-gm. sample the midribs represented about 33 per cent of the weight, while the soft tissue (that which passed through a 150-mesh sieve) constituted 48 per cent; the veins (other than the ribs), 8 per cent; and the grain, 11 per cent. Excluding the ribs, the soft tissue represented 70 per cent; the veins, 12 per cent; and the grain, 17 per cent by weight. These results are only approximate, since the primary object of the separation was to obtain a quantity of the pure grain, and the determination of the proportion of various tissues was an afterthought. It seems probable, however, that an improved method for the accurate mechanical analyses of tobacco would be highly desirable, inasmuch as its various properties doubtless depend in a large part upon the proportion of these three components of the leaf—namely, soft tissue, vein, and grain.

CHEMICAL NATURE OF GRAIN

Chemically, the grain has been supposed by Sturgis (7) and by Loew (5, p. 38–39) to be calcium oxalate, while from the fact that the grain particles produce minute explosions as fire reaches them in the process of

smoking, they have been considered by many to be composed of potassium nitrate. However, it has been shown that the grain is slowly soluble in water. This was done by allowing free-hand sections of a fermented leaf to remain in water for 4 hours and also by permitting a piece of a similar leaf to soak in water on a slide for 17½ hours. In order to show the position of the grain bodies and the idioblasts containing calcium oxalate, camera-lucida sketches were made with the aid of polarized light at the beginning of the treatment with water. In both cases, at the end of 4 and 17½ hours, respectively, no crystalline material remained in the positions of the grain bodies, where at the beginning of the experiment the bodies were highly active in polarized light. In the positions of the bodies, however, both in the sections and in the piece of leaf, the cells that had contained the crystalline material were found to be markedly distended, although upon the application of the slightest pressure their walls immediately collapsed, giving additional proof that the substances which had formerly filled the cells and held them rigid had been dissolved out. The calcium oxalate sand crystals and the scattered, single crystals referred to above remained unchanged.

In a subsequent experiment a piece of fermented leaf tobacco was dehydrated in alcohol and cleared and examined in xylol to determine the character and distribution of the grain bodies and at the same time the position and abundance of the idioblasts and the single, scattered crystals. The xylol was then removed from the piece with alcohol and the latter in turn displaced by distilled water, in a relatively large volume of which the material was allowed to remain for 24 hours at room temperature. An examination with polarized light was then made with the specimen mounted in water. In this case also it was noted that the crystalline material had disappeared from the grain bodies, but it was also evident that neither the calcium oxalate sand crystals nor the single, scattered crystals had been affected by the treatment. This was verified by dehydrating, clearing, and mounting the tissue as already described. The piece of leaf was then run back through water and allowed to stand in 50 per cent acetic acid for 48 hours and mounted and examined in the same medium. Both at this examination and after washing in water and again dehydrating and clearing, no scattered, single crystals could be found—that is to say, the only crystalline material remaining in the tissue after successive treatments with distilled water and with 50 per cent acetic acid was the sand crystals of calcium oxalate. However, these last all disappeared, leaving no optically active crystalline substance whatever in the cells after the tissue had been subjected to treatment with 50 per cent hydrochloric acid for about 17 hours.

When separated from the surrounding tissues, however, the grain bodies seemed to be less readily soluble in distilled water than when they were treated *in situ*. This is probably due to the presence of some substance in the tissues of the leaf which affects the solubility of the grain. In

every case, however, the grain-forming material was dissolved upon treatment with water for 24 hours. There were left in the cells which had formerly contained the solid crystalline substance the scattered, single crystals and also frequently calcium oxalate sand crystals an idioblast of which had been included in the group of cells which became petrified through the deposit in them of the grain-forming substance.

HYGROSCOPIC PROPERTIES

In connection with the physical and chemical characteristics of the grain may also be mentioned the results of some determinations of the hygroscopicity of some of the component parts of the leaf procured by the mechanical process already described. For these determinations four classes of air-dry material were used: (1) the leaf web—that is, the soft, parenchymatous tissue which passed through a 200-mesh sieve; (2) large veins—that is, those which, excluding the midrib, would not pass through a 20-mesh sieve; (3) smaller veins, which passed through a 30—but not an 80-mesh sieve; and (4) grain, a mixture of various sizes. Two-gm. samples of each of these classes were exposed for 50 hours side by side in open, tared Petri dishes to an atmosphere containing moisture derived from 125 gm. of granulated cigar-filler tobacco containing 26 per cent of moisture, a tight desiccator kept at about 30° C. being used as a container. The percentages of moisture absorbed by the samples were determined in the usual way, with the following results: Leaf web, 20 per cent; large vein, 17 per cent; small vein, 25 per cent; and grain, 14 per cent.

The granulated tobacco was used as a source of moisture in order to produce, as nearly as possible, natural conditions and the greatest competition between the samples and also between these and the source of moisture, at the same time insuring against the condensation of moisture within the desiccator through changes in temperature. While these results are products of work of a preliminary nature, enough has been done to indicate that the grain is the least and the small veins the most hygroscopic of the kinds of material studied. In connection with the latter it is believed that their power to absorb so large an amount of moisture may be due, at least in part, to a colloidal substance present in some of their xylem or phloem elements. This substance has the property of swelling markedly and protruding from the ends of pieces of small veins when the latter are submerged in water.

QUALITATIVE TESTS

Qualitative tests of the pure grain and its ash showed an abundance of calcium, some potash and magnesia, and a little ammonia, but only traces of oxalic, nitric, and sulphuric acids, the last of which was proved to arise chiefly from the combustion of proteids in the process of ashing.

The ash of the grain, however, contained a large amount of carbonate and therefore indicated the presence of some organic acids other than oxalic.

Aside from the solubility of the grain material in water and the detection of only a small amount of oxalic acid by ordinary qualitative means, Borodin's method (2), which consists of treating the substance to be tested with a saturated solution of the suspected substance, showed the grain to be composed of a salt or salts other than calcium oxalate.

Further, through the interest and kind assistance of Dr. F. E. Wright, of the Geophysical Laboratory of the Carnegie Institution of Washington,[1] the application of the petrographic microscope and methods to a study of the crystalline substances of the grain revealed the presence of normal calcium malate in these bodies. These methods also showed the presence of other crystalline substances, the identity of which has not yet been established. As is evident from the detailed quantitative analyses hereinafter recorded, the grain is a mixture of salts some of which may be double or even triple combinations, and the difficulties of procuring these various possible compounds in crystalline form for a comparison of their optical properties with those of the salts entering into the composition of the grain have not been surmounted at this stage of the investigation.

QUANTITATIVE ANALYSES

The data given in Table I represent quantitative determinations made on portions of the same material as that from which samples were taken for a study of the hygroscopic properties. The mineral components were determined by the official methods [2] and the organic acids by the method of Kissling.[3]

TABLE I.—*Composition of the leaf web, vein, and grain of Pennsylvania tobacco, on basis of sand-free air-dry material*

Constituents determined.	Leaf web passing through 200-mesh sieve.	Large vein (excluding midrib) not passing through 20-mesh sieve.	Small vein passing through 30-mesh but not 80-mesh sieve.	Grain of various sizes.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
Moisture	7.80	7.11	6.25	8.06
Pure ash	16.48	17.80	16.91	40.26
Potassium oxid	4.11	5.72	5.29	3.42
Calcium oxid	6.70	6.65	6.6	26.34
Magnesium oxid	1.33	1.46	1.59	3.13
Oxalic acid	3.16	2.75	3.0	.82
Citric acid	3.26	1.15	2.91	22.38
Malic acid	3.46	4.09	5.24	13.58
Total determined [a]	29.82	28.93	30.88	77.73

[1] The writer wishes to express his deep appreciation of Dr. Wright's assistance.

[2] Wiley, H. W., ed. Official and provisional methods of analysis, Association of Official Agricultural Chemists. As compiled by the committee on revision of methods. U. S. Dept. Agr. Bur. Chem. Bul. 107 (rev.), 272 p., 13 fig. 1908. Reprinted, 1912.

[3] Kissling, Richard. Beiträge zur Chemie des Tabaks. Zur Tabakanalyse. *In* Chem. Ztg., Jahrg. 28, No. 66, p. 775–776, 1 fig. 1904.

[a] Pure ash not included.

The analyses recorded in Table I show that the grain contains a very high percentage of ash, that calcium is by far the most abundant constituent, that nearly as much magnesium is present as is contained in the three other parts of the leaf taken together, but that a smaller amount of potash is found in the bodies than in any other class of leaf material studied. Of the organic acids, citric and malic are abundant in the grain, while oxalic acid is present in a much smaller percentage than in any of the other classes of material. These data indicate that the grain material must be a mixture of citrates and malates, chiefly of calcium, with some magnesium and a little potassium. Nicotin seems to enter into the composition of the grain also, since about 1.5 per cent of that substance was found in an analysis of one sample of grain. The totals of the percentages of substances determined indicate that the grain (77.73 per cent total determined) contains little material other than these substances. There remains in this case only about 22 per cent undetermined, and this must account for cellulose, nicotin, and other nitrogenous compounds, sulphur compounds, and phosphoric acid, as well as small quantities of sodium, iron, aluminum, etc., while in the three other classes these undetermined substances constitute in each about 70 per cent.

CORRELATION OF THE GRAIN WITH BURNING QUALITY

Although persons experienced in the handling of tobacco consider that a well-developed grain is an indication of good quality, particularly with reference to the burn, it seems that this is a matter of practical experience with them rather than a factor permitting definite discussion or explanation.[1] They assert that a "close-grained" leaf will burn poorly, while one possessing an "open grain" will have a greater fire-holding capacity. Recently, in the course of interviews with packers and manufacturers in Lancaster County, Pa., these observations have been found capable of substantiation by microscopic examination. The writer requested each of several practical tobacco men to select, on the character of the grain alone, what he considered to be a good-burning and a poor-burning leaf. In making this selection the leaf was stretched and while taut was allowed to pass slowly through the fingers, usually accompanied by the remark that the leaf showed an open grain or that it was close-grained. The leaves designated as poor-burning (close-grained), invariably possessed a hard texture—that is, something of the nature of the softer grades of paper—always showed poor elasticity, and rarely exhibited grain bodies on the surface. On the other hand, the leaves judged to be good-burning (showing an open grain) were comparatively soft in texture, elastic, and usually possessed grain bodies sufficiently large to

[1] See also Hayes, H. K., East, E. M., and Beinhart, E. G. (4, p. 28). The authors used seven classes to indicate the prominence of grain in considering the quality of the strains studied.

be visible to the unaided eye. It may be mentioned, however, that occasionally a leaf was found which was classed among the "open-grained" the grain of which was not apparent on the surface of the leaf, though evidently the experienced hand could detect its presence and determine its character.

The burn of the leaves thus selected was tested, always with the result predicted. A small piece was then cut from the unburned portion of each leaf at about a quarter of an inch from the point at which the fire was extinguished. These pieces were dehydrated in absolute alcohol, cleared and mounted in cedar oil, and examined microscopically with polarized light. Without exception, the good-burning leaf showed that the grain material had become well aggregated into definite bodies separated one from the other by a band of tissue free from crystalline material, save for the scattered, single crystals and occasional idioblasts containing calcium oxalate. This condition was evident even in the open-grained leaves mentioned above in which no grain was macroscopically visible on the leaf, for in those cases the bodies, though too small to cause a swelling on the surface, were definite in form and sufficiently separated to allow a zone of comparatively empty cells around each.

In the pieces from the poor-burning (close-grained) tobacco, on the contrary, the crystalline grain material proved to be scattered more or less evenly throughout the tissue, without any considerable degree of aggregation into definite bodies large enough to leave an encircling zone of empty cells around each particle. A few poor-burning samples have been found, however, in which some aggregation of grain substance had occurred, but in these the intervening tissue was filled with a mass of grain material which, for some reason, had failed to form definite bodies, thereby producing the same condition found in the poor-burning samples which were without appreciable aggregation.

From these facts it would seem that a certain degree of aggregation, with intervening tissue free from grain substance, is necessary in order that good fire-holding capacity may be assured. This suggests that the substance composing the grain bodies may have in reality a retarding effect upon the advance of fire in the tissue and that zones of grain-free cells must be present in order that a sufficient degree of heat may be generated, by their more rapid combustion, to ignite the solid grain particles. Verification of this suggestion has partially been obtained by watching through a binocular microscope the progress of the fire in the tissue. In a piece of leaf with prominent grain it was found that in the process of burning the fire line passed part way around and in some cases even beyond the centers of large grain bodies before they became ignited. It seems reasonable to suppose that the same process takes place in a leaf in which the grain particles are too small to admit of investigation in this manner and, further, that in a close-grained leaf there

is not enough grain-free tissue to act as kindling material and produce the temperature required to ignite the almost solid mass of grain substance characteristic of poor-burning tobacco.

While several theories have been set forth[1] concerning the burn of tobacco, for the most part chemical and differing from the hypothesis just advanced, an instance in favor of the present explanation is found in the case of the notoriously poor-burning 1909 crop of Pennsylvania. In this tobacco the grain substance is abundant, but it occurs in the tissue as an almost solid sheet, showing very poor aggregation into definite grain bodies (Pl. 17, fig. B). Again, flue-cured tobacco, which is always considered a poor-burning type, is entirely without grain aggregates. In this tobacco there is a small amount of grain substance (Pl. 17, fig. C) compared with cigar types, and it appears to be deposited very rapidly in the form of a haze of minute crystals throughout the tissues of the leaf. It is thought that the rapid-curing method employed with this type is responsible for this condition, inasmuch as the same kind of tobacco, grown in the same region and air-cured instead of being subjected to heat, shows to some degree the aggregation of the grain substance into definite bodies. In this connection it may be said also that Connecticut tobacco (Pl. 17, fig. D), which as a rule shows an excellent burn, also possesses a high degree of aggregation of the grain substance.

In connection with some investigations concerning the cause of the defects in the burning quality of York County tobaccos jointly pursued by the Office of Tobacco and Plant Nutrition Investigations and the Pennsylvania Agricultural Experiment Station[2] working cooperatively, a certain degree of correlation of grain formation and burning quality has been found. The tobacco was grown at Red Lion, Pa., during the seasons of 1913 and 1914 in duplicate plots upon which nine different fertilizer treatments, identical for the two years, were used to determine their influence upon the burning quality of the product. The material used for microscopical investigations was comparable to that upon which the burn and other factors were determined. In preparing the material for examination with the microscope, disks were cut from the leaves

[1] Garner discusses (3) the more important contributions concerning the burn of tobacco. Of the two physical theories thus far advanced, the one sought (1) to correlate the burning quality of tobacco, at least in part, with such anatomical features of the leaf as the number of rows of cells, the size of intercellular spaces, etc., while the other attributes (6) a beneficial influence to the action of potash salts of the organic acids in swelling to many times their original bulk and thereby yielding a porous mass of finely divided carbon when decomposed by heat. The chemical data given herewith do not support the latter theory, since the organic acids are largely localized in the grain, and only a small portion of the potassium is present in these bodies. This indicates that the greater portion of the potassium in the leaf is not in combination with the organic acids. No experimental work has been done by the writer concerning the former theory, though it is believed that in view of the results published herein and the fact that in cigar tobaccos the intercellular spaces are obliterated and the cell walls collapsed during the fermentation process little ground would be found for its substantiation.

[2] A report of this cooperative work by Dr. William Frear, Chemist and Vice Director of the Pennsylvania Agricultural Experiment Station, will appear in the annual reports of that institution for 1913–14 and 1914–15.

after fermentation and these were dehydrated in absolute alcohol, cleared in xylol, and mounted in Canada balsam.[1]

In ordinary transmitted light the samples showed no marked variations one from the other. The use of polarized light, however, revealed striking differences in the degree to which the grain substance had become aggregated, and it was found that these differences seemed to remain fairly constant between the good-burning samples on the one hand and the poor-burning samples on the other. In order that the condition found might be expressed in some form more concise than by descriptive terms, a system of scoring the degree of aggregation was adopted. By using a maximum perfect score of 10 points, convenient minor classes occurred as follows: "Good," 8 points; "fair," 6 points; "poor," 4 points; and "very poor," 2 points. A detailed score (Table II) of a poor-burning sample will best serve to illustrate the method used.

TABLE II.—*Detailed score of poor-burning sample from Plot I, Red Lion, Pa., 1913*

Disk No.	Grain.	Score.
1	Poor aggregation; small amount; haze of single and cryptocrystalline crystals	4
2	Poor aggregation; small aggregates; flaky spherites, cryptocrystalline haze	3
3	Very poor aggregation; small amount; fine cryptocrystalline haze with larger singles	2
4	Very poor aggregation; dense, hazy mass	2
5	Poor aggregation; similar to disk 4, though less dense	3
6	No aggregation; spherites and cryptocrystalline haze	1
7	Very poor aggregation; similar to disk 4	2
8	No aggregation; fine, thin cryptocrystalline haze	0.5
9	Poor aggregation; dense; a few flaky and a few black superficial aggregates	2.0
		19.5
	Divided by 9, score is	2.16

It will be noted in the score that the word "spherites" is used in the description of some of the samples. These are of much more frequent

[1] Of the 1913 material only the two best and the two poorest-burning samples were studied in detail, while the samples from all the fertilizer treatments used in 1914 were subjected to an equally thorough investigation. The microscopical data here presented represent, for the 1913 material, detailed examinations of disks cut from each of 18 representative leaves from each of the four fertilizer treatments after the fermentation process was completed. In 1914, however, the leaves were selected in the field before the plants were harvested. In this case three leaves from each of six average plants in each treatment were sampled in the field by removing a small disk of tissue from each leaf selected at a point equidistant from the midrib and the margin and midway between the base and apex. These disks were killed in absolute alcohol and preserved for future study in the same liquid. The leaves thus sampled were the eleventh, twelfth, and thirteenth below the point of topping, which point in all of the plants bore, as nearly as possible, the same relation to the developing flowers. These same leaves, having been tagged in the field, were used later in a preliminary study of the development of the grain and, after fermentation, for determining the character of the fully developed grain together with the fire-holding capacity and certain chemical analyses.

occurrence in poor-burning than in good-burning tobacco, as are also large, single crystals of grain substance and aggregates composed of large, platelike or flaky crystals. The cryptocrystalline haze is also much more prominent in the tissues between the relatively few definite grain bodies in the poor-burning tobacco. It will also be noted that no account is taken of the calcium oxalate or the single, scattered, acetic-acid-soluble crystals. The result of this method and the correlation of this result with apparently related factors are seen in Tables III and IV and figures 1 and 2. Corresponding data relative to the potash and chlorin content of the tobaccos are also included in the tables.

TABLE III.—*Data relative to the burning quality of tobacco from fertilizer treatments at Red Lion, Pa., crops of 1913 and 1914*

Treatment.	Burn score (maximum=20 points).[a]	Fire-holding capacity in cigar.[a]	Grain aggregation (Maximum=20 points. Original score × 2).[b]	Potash content.[a]	Chlorin content.[a]	Potash-chlorin ratio.[a]	Burning quality.
1913							
		Min. sec.		*Per cent.*	*Per cent.*		
I	13.3	4 25	5.4	3.7	1.78	2.3	Poor.
III	18.0	5 37	13.2	4.01	.56	7.1	Good.
VII	17.5	6 7	12.8	4.14	.64	6.7	Do.
IX	11.0	4 22	7.3	3.44	2.66	1.3	Poor.
1914							
I	14.0	4 10	5.7	3.61	1.38	2.6	Poor.
III	18.0	8 0	11.1	3.42	.26	13.15	Good.
VII	18.0	6 15	9.7	3.57	.23	15.5	Do.
IX	12.0	3 45	4.3	3.34	2.44	1.36	Poor.

[a] Data furnished from report of cooperative work by Dr. William Frear, Chemist and Vice Director of the Pennsylvania Agricultural Experiment Station. The burn score and fire-holding capacity in the cigar were determined by Mr. Otto Olson, Assistant in Tobacco Investigations, Bureau of Plant Industry, and the determinations of the potash and chlorin content and the potash-chlorin ratio were made by Mr. E. S. Erb, Assistant Chemist, Pennsylvania Agricultural Experiment Station.

[b] Data obtained by the writer and included in the report of Dr. W. W. Garner on the portion of the cooperative work undertaken by the Office of Tobacco Investigations, Bureau of Plant Industry.

In Tables III and IV and figures 1 and 2 the fire-holding capacity of the cigar has reference to the length of time a cigar made entirely of the tobacco in question remains lighted. The burn score includes the fire-holding capacity, amount of charring, character of ash, etc.

In determining the fire-holding capacity of the open leaf the writer ignited separately each leaf of the sample with the glowing end of a piece of punk, and the number of seconds during which the fire glowed in the larger portion of the burning area was taken as the fire-holding capacity.

TABLE IV.—*Data relative to the burning quality of the tobacco grown on fertilizer plots at Red Lion, Pa., in 1914*

Treatment.	Fertilizer applied per acre. Kind.	Quantity.	Burn score (maximum 20 points).[a]	Fire-holding capacity in cigar.[a]	Fire-holding capacity, punk-stick method.[a]	Grain aggregation score (maximum 20 points. Original score × 2.[a])	Potash content.[a]	Chlorin content.[a]	Potash-chlorin ratio.[a]
		Pounds.		*Min. sec.*	*Sec.*		*Per ct.*	*Per ct.*	
I	Manure	20,000	14.0	4 10	8.0	5.7	3.61	1.38	2.6
II	Manure	20,000	16.0	5 0	6.2	6.0	3.85	.91	4.2
	Dissolved rock	343							
	Potassium sulphate	100							
III	Cottonseed meal	1,485	18.0	8 0	16.8	11.1	3.42	.26	13.15
	Potassium sulphate	258							
IV	Cottonseed meal	1,485	17.5	5 22	14.8	7.1	3.17	.18	17.61
	Dissolved rock	190							
	Potassium sulphate	258							
V	Cottonseed meal	1,485	17.0	5 45	14.2	8.1	3.75	.19	19.73
	Dissolved rock	372							
	Potassium sulphate	258							
VI	Cottonseed meal	1,485	17.5	5 7	19.8	8.1	4.06	.23	17.7
	Potassium sulphate	129							
	Potassium carbonate	112							
VII	Cottonseed meal	1,485	18.0	6 15	20.5	9.7	3.57	.23	15.5
	Precipitated bone	136							
	Potassium sulphate	258							
VIII	Cottonseed meal	1,485	17.0	5 52	10.1	7.0	3.47	.23	15.08
	Potassium carbonate	224							
	Dissolved rock	190							
IX	Cottonseed meal	1,485	12.0	3 45	5.4	4.3	3.34	2.44	1.36
	Dissolved rock	372							
	Potassium chlorid	258							
Average for treatments I, II, IX			14.0	4 18	6.5	5.3	3.6	1.58	2.7
Average for treatments III to VIII, inclusive			12.5	6 35	16.0	8.5	3.57	0.22	16.46

[a] Same as for Table III.

As is illustrated by figures 1 and 2, representing the data included in Tables III and IV, respectively, the curves for the burn, the fire-holding capacity in the cigar, and the grain aggregation show a marked parallelism, although in the fire-holding capacity determined on single leaves the second highest point in the curve does not correspond to either of the principal maxima in the three other curves.

While the relative values representing the physical factors determined for the poor-burning samples correspond in a general way with Dr. Frear's

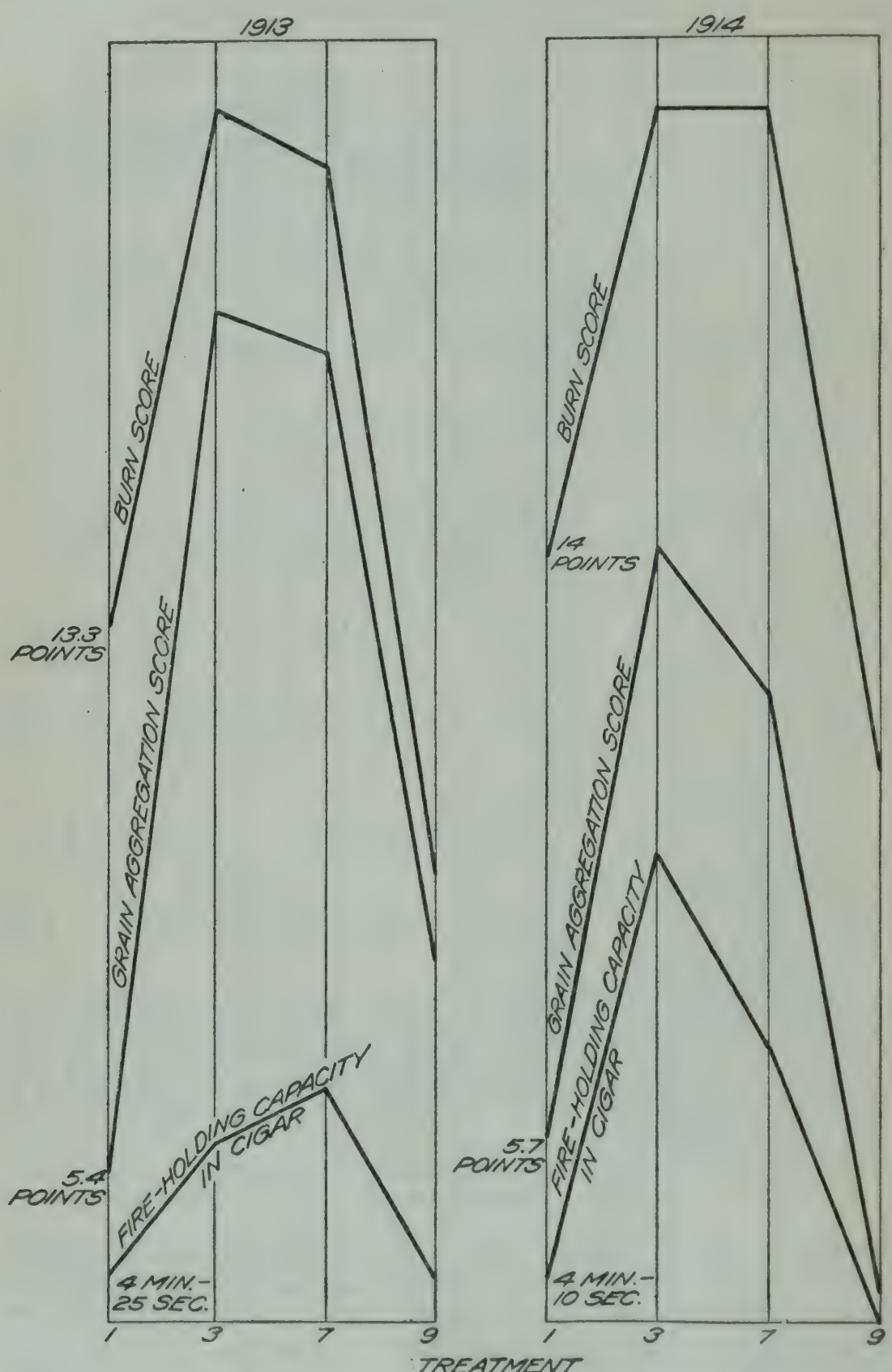

FIG. 1.—Curves plotting data relative to the burning quality of tobacco from fertilizer treatment at Red Lion, Pa., for crops of 1913 and 1914. Data taken from Table III.

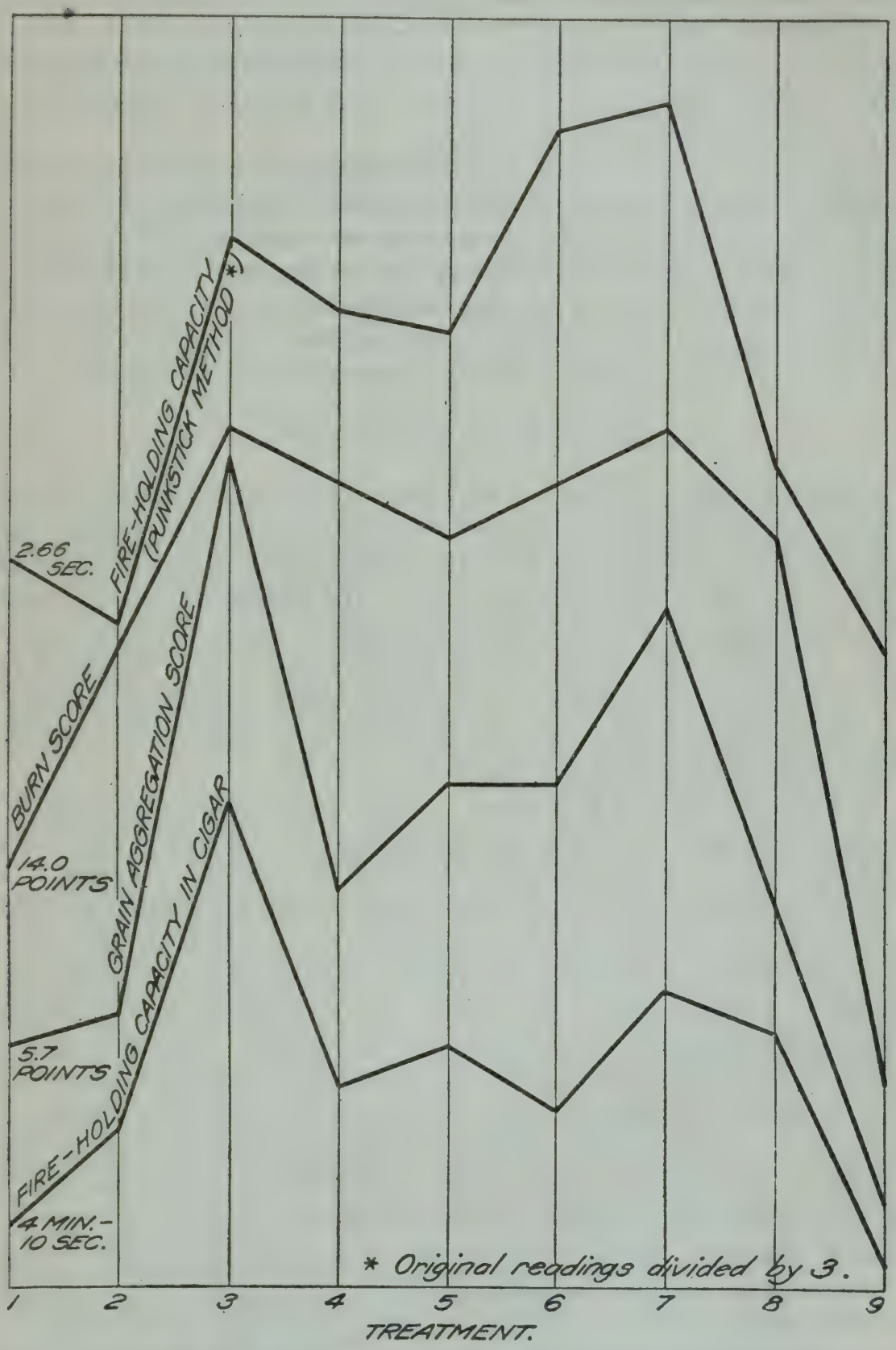

FIG. 2.—Curves plotting data relative to the burning quality of the tobacco grown on fertilizer plots at Red Lion, Pa., in 1914. Data taken from Table IV.

potash-chlorin ratios and show an inverse relation to his chlorin-content figures, this relation does not hold for the good-burning samples, except in the case of the Treatment VI previously referred to. The relative inferiority of the poor-burning samples in this series is doubtless traceable in part to their high content of chlorin, which element is commonly reputed to be antagonistic to combustion.

Though a more or less definite correlation seems to exist in the tobacco studied between the burning qualities and grain aggregation, for which a tentative explanation has been advanced, no reason is evident at this time for the apparent relation between the low degree of aggregation and the high chlorin content of the poor-burning samples. It is hoped that future work may throw some light upon this phase of the problem as well as upon other possible factors affecting grain formation in tobacco.

DEVELOPMENT OF THE GRAIN

While the opportunity has not been offered for a thorough study of the process of formation and aggregation of the grain in the leaf, preliminary investigations upon material referred to in footnote on page 279, as well as upon various kinds of tobacco observed in the laboratory, indicate that the grain substance is not present in crystalline form in the living, green leaf. These observations prove that the formation and development of the grain bodies are concomitant with the process of curing, and there are indications that the aggregation of the grain substance continues during fermentation (compare Pl. 17, fig. A, E, G).

At Red Lion, Pa., on September 22, 1914, four days after harvesting, samples of tobacco were becoming flecked near the margins of the leaves with the yellow mottling characteristic of the early stages of curing.

In this condition microscopic examination showed the absence of grain bodies and also that the protoplasmic contents of the cells were still intact. In the later stages of yellowing and with the apparent death and disorganization of the protoplasm, just before the development of the brown color, highly refractive droplets appeared in the cells. Upon attempting to dehydrate a piece of such a leaf in alcohol minute crystals appeared in these droplets. In the next stage—that is, at about the first indication of a brown color in the leaf—the droplets had enlarged and become decidedly viscid, and each showed upon examination with polarized direct sunlight conspicuous crossed extinction bands and presented the appearance of spherites. Upon dehydration at this stage they developed no small crystals. As the browning progressed, certain of the bodies seemed to differentiate within themselves, without artificial dehydration, a nucleus of crystals of definite though not identifiable form. The substance of the droplets in which no crystals had formed seemed to migrate toward those which had produced crystal nuclei, increasing by accretion the crystalline mass until groups of several cells each had become literally

petrified and formed a solid grain body. Definite grain was formed in leaves of average maturity in nine days after the tobacco was harvested.

It is thought that the migration of the still viscid substance was brought about by diffusion, through the dead and permeable protoplasm, set up by a lowering of concentration in the immediate vicinity of the developing crystal nuclei. Under ideal curing conditions it is probable that this process results in the aggregation of all, or nearly all, of the grain-forming substance into definite bodies, leaving around each a zone of cells largely free from that material. It is believed that under these conditions the number of grain bodies developed in a given area is dependent upon the number of crystal nuclei formed. The factors which determine the number and distribution of the latter are not known. It is thought, however, that the rate of desiccation and the degree of maturity of the curing leaves may be of importance in this respect.

The behavior of the grain material in the flue-cured tobacco seems to support the idea that the rate of drying is a factor in grain development. In the completely cured leaf of this type the substance which would have formed more or less definite grain bodies under ordinary air-curing conditions apparently has been thrown down in crystalline form by the rapid curing and drying characteristic of the method, in the first droplet stage referred to above, resulting in a haze of minutely crystalline material in all the cells. Even Connecticut tobacco, when cured very rapidly ("hayed-down"), frequently shows poor burning qualities. Again, in the poor-burning crop of tobacco produced in Pennsylvania in 1909, which was a "dry-weather crop," the grain material failed to become well aggregated. It seems probable that either a low percentage of water in the tissues or an abnormally large quantity of grain-forming substance in solution in the cell sap at the time of harvesting, or both, must have resulted in the formation of an unusually large number of crystal nuclei in a given area, especially if weather conditions were such as to cause rapid dessication in the early stages of curing. In the later stages, then, opportunity for the development of zones of cells free from grain substance would have been limited by the closeness together of the crystal nuclei. Indeed, samples of this crop have been seen in which the grain substance seemed to have been thrown down in practically every cell, much in the manner of that of flue-cured tobacco, though greater in amount. Of interest to note in passing is the fact that the burning properties of this crop, much of which is still in storage, are very gradually improving. It seems probable that this may be due in part to the slow aggregation of grain material rather than to the loss through aging of some substance injurious to the burning quality.

The leaves upon which the observations were made at Red Lion, Pa., were sampled after the curing had been completed, and it was found that the grain throughout the leaf was in practically the same condition

as had obtained near the outer edges of the leaves on the ninth day of curing. Examination after the same leaves had been fermented, however, showed that the grain was much more pronounced on the surface, and the microscope revealed a greater degree of aggregation than had existed at the end of the curing process. It is believed that with the high water content of the fermenting tissue the grain substance is gradually put in solution, particularly in the case of the smaller bodies, and that further grain aggregation and development of intervening zones of comparatively empty cells is thereby made possible.

SUMMARY

(1) The grain of cigar tobacco consists of hard bodies which, if sufficiently large, cause the surface of the leaf containing them to present a pimply appearance. This grain, in connection with other intimately related properties of the leaf, constitutes an important factor in determining the value of cigar tobacco.

(2) Each body consists of from one to several leaf cells distended with a mass of crystalline substance. They are most prominent microscopically when examined with polarized light. Although visible in ordinary transmitted light, owing to the concentration in them of a large part of the brown coloring matter of the leaf, the details of their structure are more apparent when the former method of examination is used.

(3) Based upon microscopic features, five forms or types of grain are recognized, though their significance is a matter still to be investigated.

(4) Two other kinds of crystalline material are found in the tobacco—namely, cryptocrystalline calcium oxalate, contained in certain cells in the various tissues of the leaf; and single, small, prismatic crystals scattered evenly throughout the leaf, one in nearly every cell of the mesophyll and epidermis.

(5) A mechanical method resulted in the separation of the grain bodies from the other portions of the leaf and gave an approximate mechanical analysis. This analysis showed roughly the percentages by weight of leaf web, veins, and grain.

(6) Chemical analyses proved that the grain is composed chiefly of calcium, with a little magnesium and potassium, in combination with citric and malic acids rather than with oxalic acid. One of the salts, normal calcium malate, was identified by petrographic methods. Determinations of the hygroscopic properties of the component parts of the leaf separated by mechanical means indicate that the grain is not responsible for the marked hygroscopic properties of tobacco, since it absorbed the least water from a moist atmosphere. The small veins showed the greatest hygroscopicity.

(7) The grain bodies of tobacco are developed in the course of post-mortem changes which take place during the process of curing and con-

tinue during fermentation. A microscopically visible change consists of a more or less complete aggregation of the grain-forming substance of all the cells into certain groups of cells. The factors determining the location of these groups are unknown.

(8) In the tobacco studied a correlation was found between the grain and burning properties. It is believed that the substances contained in the grain bodies are injurious to the burn and that the quality of the latter is dependent upon the degree to which the former are aggregated into definite bodies sufficiently separated, one from the other, to permit a considerable fire-carrying zone of cells, emptied of grain material, around each. The influence of the degree of aggregation of the grain substance upon the color, texture, and elasticity of the leaf has not yet been thoroughly investigated.

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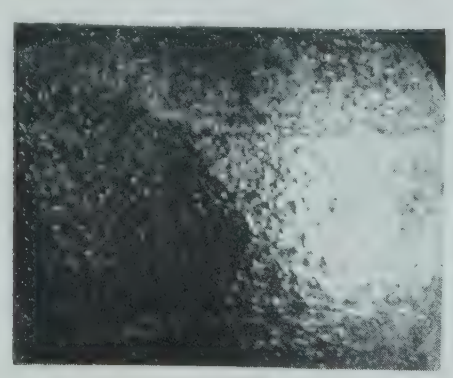
PLATE 15

Fig. A, B.—Well-cased tobacco leaves stretched over the closed end of a test tube; showing very pronounced grain development. × 2.

Fig. C.—A portion of a cigar wrapped with a leaf containing very coarse grain. × 2.

Fig. D.—The same as figure C, but after a portion of the cigar had been smoked, showing the white pimples in the ash produced by the burning and swelling of the grain bodies. × 2.



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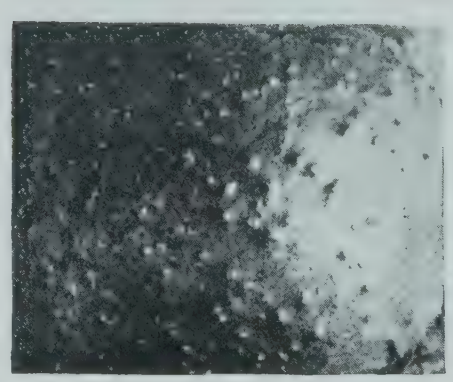

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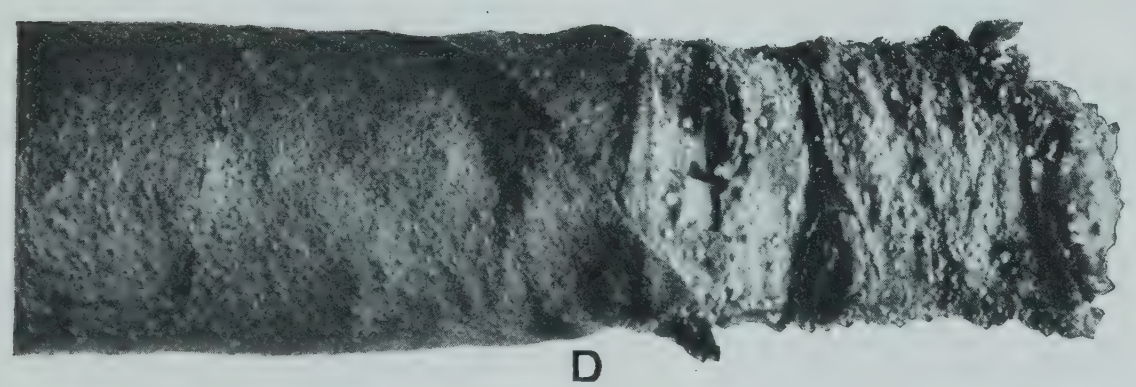

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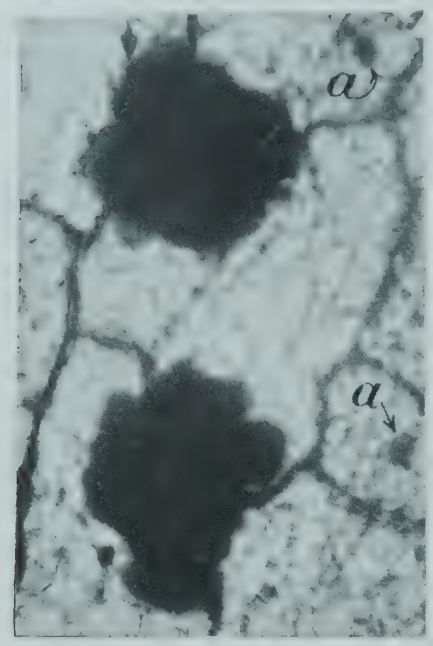

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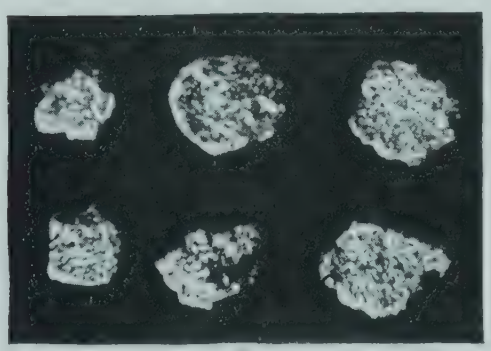

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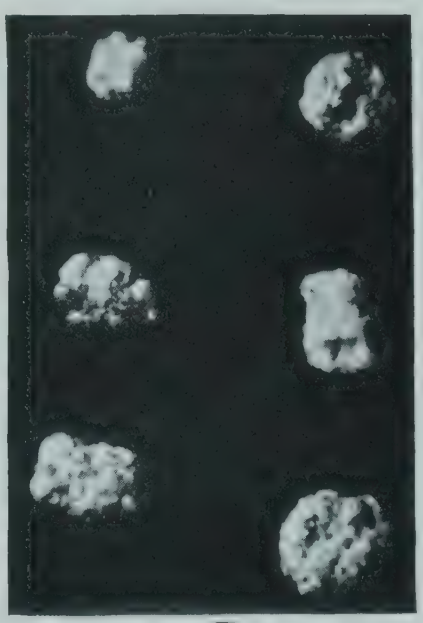


D

E

F

PLATE 16

Fig. A.—Grain bodies of Connecticut Broadleaf tobacco as seen in ordinary transmitted light. *a*, Idioblasts containing sand crystals of calcium oxalate. × about 300.

Fig. B.—Representative grain bodies of class 1. × 19.5.

Fig. C.—Representative grain bodies of class 2. The high lights show the positions of the distended epidermal cells. × 19.5.

Fig. D.—Representative grain bodies of class 3. × 19.5.

Fig. E.—Representative grain bodies of class 4. × 19.5.

Fig. F.—Grain substance in the form of minute spherites. Photographed with polarized light. × 280.

PLATE 17

Fig. A.—Green tobacco leaf from Treatment I, Red Lion, Pa., 1914, killed in absolute alcohol and showing idioblasts of calcium oxalate and minute, scattered, single crystals of an undetermined substance, but no grain. Photographed with polarized light. × 65.5.

Fig. B.—Representative sample of the poor burning 1909 Pennsylvania tobacco. Photographed with polarized light. × 65.5.

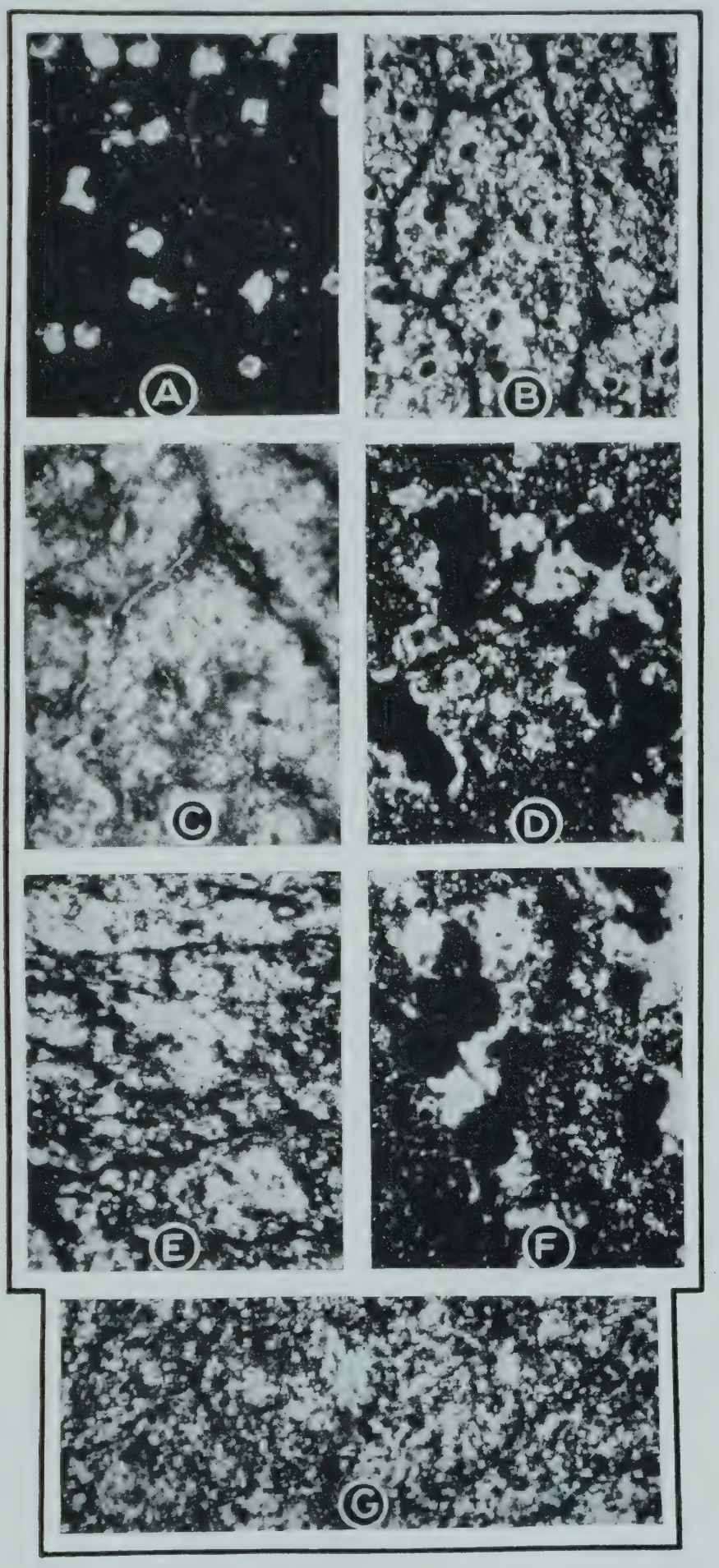
Fig. C.—Flue-cured tobacco. Poor-burning. Photographed with polarized light. × 65.5.

Fig. D.—Connecticut Broadleaf tobacco. Good burning. The brown coloring matter concentrated in the grain partially masks their structure in some cases. Photographed with polarized light. × 65.5.

Fig. E.—Fermented tobacco from Treatment I, Red Lion, Pa., 1914. Poor burning. Photographed with polarized light. × 65.5.

Fig. F.—Fermented tobacco from Treatment III, Red Lion, Pa., 1914. Good burning. The brown coloring matter concentrated in the grain partially masks their structure in some cases. Photographed with polarized light. × 65.5.

Fig. G.—Tobacco from Treatment I, Red Lion, Pa., 1914. Cured only. Photographed with polarized light. × 65.5.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HOST PLANTS OF THIELAVIA BASICOLA

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The increasing economic importance of *Thielavia basicola* Zopf as a root parasite of certain cultivated plants has led to a desire for more complete information regarding its range of host plants. This knowledge is important not only from the standpoint of the effect upon the hosts themselves but in the relation of the use of these host plants in rotation with other crops susceptible to attack by the fungus. *T. basicola* appears to be primarily a parasite of leguminous plants, and the common use of these in rotation may prove to be unprofitable for certain crops. The benefit derived from the added fertility in the soil may be entirely offset in certain instances by the injury done as a result of maintaining the fungus in the soil through the use of host plants in rotation.

From a mycological point of view, considerable interest may also be attached to the hosts of *T. basicola*. The earlier botanists who observed this fungus were in some doubt as to the parasitic nature of the organism. This view has persisted to some extent, and the fungus is still believed by some to be purely superficial in its mode of life and not ordinarily injurious when present. On the other hand, some of the later investigators have not only convinced themselves of the parasitism of the organism but also of a high degree of pathogenecity, while some have gone so far as to suppose the fungus capable of attacking almost any plant under favorable conditions (11).[2] It is fairly clear to the writer that this difference in opinion is due in a large measure to not only the species but the variety of host plant under observation. Considerable emphasis has also been laid upon the conditions necessary for disease to be developed by this fungus, leading to the supposition that certain unknown special conditions are necessary and that infection is difficult to obtain. Although this may be true within certain limits, it has been found that infection could be repeatedly and easily produced with the more susceptible plants. Where the writer has experienced difficulty in obtaining infection on certain host plants, it has been attributed rather to resistance or immunity in the

[1] This investigation was carried on in cooperation with the Bureau of Plant Industry, Department of Agriculture.

[2] Reference is made by number to "Literature cited," p. 299–300.

plant itself than to lack of favorable conditions for fungus attack. Plants of known susceptibility were at all times grown as controls in the infection experiments and served as a good basis for such conclusions.

HISTORICAL REVIEW

Berkeley and Broome (2, p. 461) in 1850 first described the fungus now known as *T. basicola*. These authors found it at the base of stems of *Pisum sativum* and *Nemophila auriculata*. The fungus was apparently not again noted until 1876, when Zopf (23) reported it from Germany on *Senecio elegans* and Sorokin (20) from Russia on horseradish (*Cochlearia armoracia*). Massee (10) described a new fungus in 1884 which he found on decaying leaves of *Blysmus compressus* and named it *Milowia nivea*. This fungus he considers in a later paper (11) as having been *T. basicola*. From descriptions and drawings of this earlier described form, however, it may be seen that there is some room for doubt as to the identity of these two forms.

In 1891 Zopf (24) again published upon the occurrence of *Thielavia basicola* and noted that it was especially common on leguminous plants, adding the following new hosts: *Lupinus angustifolius*, *L. albus*, *L. thermis*, *L. luteus*, *Trigonella coerulea*, and *Onobrychis crista-galli*. In the same year Thaxter (22) made the first report of the occurrence of *T. basicola* in America, finding it on the violet. Sorauer (19) in 1895 reported the fungus as causing a disease of the roots of the cyclamen. Peglion (13), working in Italy, in 1897 was first to record the parasitism of *T. basicola* on *Nicotiana tabacum*. Killebrew (8, p. 162), however, as early as 1884, described the symptoms of a root disease of tobacco in Pennsylvania, which was undoubtedly due to *T. basicola*, though no causal organism was named in the description. Selby (15, p. 228) has noted *T. basicola* as occurring upon the roots of *Begonia rubra*, following the nematode disease, and later found it causing a rootrot of *Catalpa speciosa* (17, p. 384, 447). Smith (18, p. 35–38) in 1899 added two new hosts, *Gossypium herbaceum* and *Vigna sinensis*. Van Hook (16, p. 96) was first to note *T. basicola* upon *Panax* (*Aralia*) *quinquefolium*. Aderhold (1) in 1905 found *T. basicola* on begonia and carried on infection experiments with pure cultures of the organism. He obtained slight infections on *Scorzonera hispanica*, *Daucos carota*, *Apium graveolens*, and *Beta vulgaris* and better development on *Lupinus angustifolius* and *Phaseolus vulgaris*. He concluded from his experiments that *T. basicola* was only a weak parasite.

Kirchner (9) includes two previously unreported species as hosts, *Phaseolus multiflorus* and *Nicotiana rustica*. Since no reference to personal observation or infection experiments could be found, it is presumable that these are not authentic host plants, especially in view of the facts noted later.

Gilbert (7) in 1909 added three new hosts: *Linaria canadensis*, *Oxalis corniculata*, var. *stricta*, and *Trifolium repens*. Chittenden (5) in 1912 reported *T. basicola* as a parasite of sweet peas. Massee (11) in the same year added to the list of host plants a species of Cypridpedium, *Aster* spp., and *Capsella bursa-pastoris*.

Rosenbaum (14) published in 1912 on infection experiments with *T. basicola*, and by cross-inoculation experiments found that the species of Thielavia on tobacco, cotton, and ginseng were identical.

O'Gara (12) in 1915 found *T. basicola* causing a disease of *Citrullus vulgaris* in Utah. This is the first report of the fungus on a member of the cucurbit family. Recently Burkholder (3) has noted the fungus on *Trifolium pratense*, *Trifolium hybridum*, and *Medicago sativa*.

METHOD OF WORK

The investigation of the host plants of *T. basicola* was undertaken primarily to corroborate, so far as possible, the hosts reported by earlier investigators, as in the majority of instances this had not been done. As it was deemed important to know something of the possible relation of the fungus to our agricultural plants, these were for the most part included in the earlier tests; but later the trials were made to include as many species as obtainable of the more susceptible families of plants. In this way about 200 species of plants have been grown on infected soil. The work was carried on almost entirely in the greenhouse. The seeds or plants were sown or transplanted into the infected soil, and optimum conditions maintained, so far as possible, especially as regarded the moisture content of the soil. The work was done partly at Arlington, Va., in cooperation with the Office of Tobacco Investigations, Bureau of Plant Industry, and partly at the Wisconsin Agricultural Experiment Station at Madison. In the Arlington greenhouses a fairly heavy clay loam soil from a tobacco field on the farm was used. This soil was taken from a spot in the field known to be badly infected with the rootrot caused by *T. basicola*. At Madison a greenhouse soil containing considerable vegetable matter was infected with soil from a tobacco field, where this rootrot had been occurring annually in recent years. On the whole, the conditions were such as might occur outdoors during a season of high precipitation. Recording soil and air thermometers showed temperatures ranging ordinarily between 18° and 25° C. in the soil and 20° to 30° C. in the air. The data were taken largely from infection on seedlings, as this was naturally an advantage in examining minutely the root system of a large number of plants. Since the age of the individual roots is considered a greater factor than that of the entire plant in determining the occurrence of infection, it is believed the results would be comparable if older plants were used.

At intervals of about five days after the plants were well above the ground one or more plants were carefully removed from the soil with as much root system as possible. The soil was then washed off the roots, after which they were usually placed immediately in 80 per cent alcohol and transferred to the laboratory, where microscopical examination of any spots or lesions occurring on the roots were made. Where *T. basicola* was found without difficulty, three to five examinations only were made; but where it could not be found on reported or suspected hosts, the roots of 10 to 15 plants were carefully examined, except in a few rare instances where this number of plants could not be obtained.

EXPERIMENTAL RESULTS

The results obtained are presented largely in the following tables. The host plants are separated into those reported by earlier workers (Table I) and the new hosts (Table II). For convenience in reference the authority for the first report and the country and year in which reported are given. These columns are followed by the results obtained with the various plants in the present experiments, giving at the same time the approximate degree of susceptibility, as nearly as could be determined by the amount of infection obtained. In the same way these results are given for the new hosts reported here. From Table I it may be seen that out of the 39 host plants previously reported the parasitism of *T. basicola* on 25 of these has been corroborated. Of the remaining 14 species infection could not be obtained on 7 species. Seeds or plants of 7 species have not been secured up to this time, but infection in most of these cases was obtained upon closely related species, indicating at least that the unobtainable species are for the most part probably susceptible to attack. Sixty-six new species of plants have been added to those already reported and corroborated as host plants of *T. basicola*. As will be seen from the list, these are largely in the leguminous, solanaceous, and cucurbitaceous families. Although the plants tested were largely representatives of these families, a number of species of other families have been included in the tests, especially species of the Compositae, Gramineae, and Rosaceae. It is fairly safe to conclude from these tests that the latter families are generally immune from attack by *T. basicola*.

TABLE I.—*Host plants of Thielavia basicola reported before the present investigation*

Host plant.	Authority.	Locality.	Year.	Susceptibility.
1. Leguminosae:				
Pisum sativum	Berkeley and Broome (2).	England	1850	Low.
Onobrychis cristagalli.	Zopf (24)	Germany	1891	Medium.
Trigonella coerulea	do	do	1891	Do.
Lupinus angustifolius.	do	do	1891	Plants not obtained.
Lupinus thermis	do	do	1891	Do.
Lupinus albus	do	do	1891	Slight.
Lupinus luteus	do	do	1891	Medium.
Vigna sinensis	Smith (18)	United States	1899	Do.
Phaseolus vulgaris	Aderhold (1)	Germany	1905	Low.
Phaseolus multiflorus	Kirchner (9)	do	1906	None.
Trifolium repens	Gilbert (7)	United States	1909	Low.
Lathyrus odoratus	Chittenden (4)	England	1911	Slight.
Medicago sativa	Burkholder (3)	United States	1916	Low.
Trifolium pratense	do	do	1916	Do.
Trifolium hybridum	do	do	1916	Do.
2. Solanaceae:				
Nicotiana tabacum	Peglion (13)	Italy	1897	High; low.
Nicotiana rustica	Kirchner (9)	Germany	1906	None.
3. Cucurbitaceae:				
Citrullus vulgaris	O'Gara (12)	United States	1915	Low.
4. Miscellaneous families:				
Viola odorata	Thaxter (22)	do	1891	High.
Aralia quinquefolia	Van Hook (16)	do	1904	Medium.
Gossypium herbaceum	Smith (18)	do	1899	Do.
Nemophila auriculata	Berkeley and Broome (2).	England	1850	Plants not obtained.
Linaria canadensis	Gilbert (7)	United States	1909	Low.
Begonia rubra	Selby (15)	do	1896	Plants not obtained.
Begonia (tuberhybrida?).	Aderhold (1)	Germany	1905	Do.
Oxalis corniculata, var. *stricta.*	Gilbert (7)	United States	1909	Slight.
Catalpa speciosa	Selby (17)	do	1910	Do.
Senecio elegans	Zopf (23)	Germany	1876	Medium.
Aster sp	Massee (11)	England	1912	Low.
Scorzonera hispanica	Aderhold (1)	Germany	1905	None.
Pastinica sativa	Taubenhaus (21)	United States	1914	Do.
Cochlearia armoracia	Sorokin (20)	Russia	1876	Slight.
Capsella bursa-pastoris.	Massee (11)	England	1912	Do.
Cypripedium sp	do	do	1912	Plants not obtained.
Cyclamen sp	Sorauer (19)	Germany	1895	Slight.
Blysmus compressus	Massee (11)	England	1912	Plants not obtained.
Apium graveolens	Aderhold (1)	Germany	1905	None.
Daucus carota	do	do	1905	Do.
Beta vulgaris	do	do	1905	Do.

TABLE II.—*New host plants of Thielavia basicola*

Host plant.	Susceptibility.
1. Leguminosae:	
Arachis hypogaea	Medium.
Astragalus sinicus	Do.
Cassia chamaecrista	Do.
Cytisus scoparius	Low.
Desmodium tortuosum	Slight.
Dolichos lablab	Do.
Galactia sp.	Low.
Glycine hispida	Do.
Lens esculenta	Medium.
Lespedeza striata	Low.
Lotus corniculatus	Do.
Lotus villosus	Do.
Lupinus hirsutus	Medium.
Medicago denticulata	Low.
Melilotus alba	Do.
Melilotus indica	Medium.
Ornithopsus sativus	Low.
Onobrychis viciaefolia	Slight.
Phaseolus acutifolius	Medium.
Robinia pseudoacacia	Do.
Sclotis chinensis	Low.
Strophostyles helvola	Medium.
Trifolium incarnatum	Do.
Trigonella foenum-graecum	Low.
Tephrosia virginiana	Do.
Ulex europaeus	Slight.
Vicia villosa	Do.
1. Leguminosae—Continued:	
Vicia faba	Slight.
2. Solanaceae:	
Datura metel	Medium.
Datura stramonium	Low.
Datura tatula	Slight.
Datura cornucopia	Do.
Nicotiana glauca (Gratz)	Low.
Nicotiana silvestris (Speg.)	Medium.
Nicotiana sanderae (Sander.)	Low.
Nicotiana repanda (Willd.)	Do.
Nicotiana atropurpurea	High.
Nicotiana langsdorffii (Weinm.)	Low.
Nicotiana chinensis (Fisch)	High.
Nicotiana macrophylla (Lehm.)	Low.
Nicotiana glutinosa (Linn.)	High.
Nicotiana calyciflora[a]	Medium.
Nicotiana latterrima (Mill.)	High.
Nicotiana alta (Link and Otto).	Medium.
Nicotiana angustifolia (R. and P.)	Do.
Nicotiana longiflora (Cav.)	Low.
Petunia (*hybridia*?)	Slight.
Solanum carolinense	Do.
3. Cucurbitaceae:	
Cucurbita maxima	Low.
Cucurbita pepo	Do.
Cucumis melo	Do.
Cucumis sativus	Slight.
Cucurbita moschata	Low.
Cucumis acutangulus	Do.
Cucumis flexuosus	Do.
4. Miscellaneous families:	
Viola tricolor	Slight.
Begonia semperflorens	Do.
4. Miscellaneous families—Continued:	
Nemophilia aurita	Medium.
Nemophilia insignis	Low.
Linaria cymbalaria	Do.
Linaria maroccana	Slight.
Paphiopedilum grossianum	Do.
Portulaca oleracea	Low.
Ipomoea coccinea	Slight.
Phlox drummondii	Do.
Papaver nudicaule	Do.

[a] The validity of these species may be questioned.

Negative results have also been obtained with a number of species in the most susceptible families. In the legume family these apparently immune species may be represented by *Phaseolus multiflorus*, *Vicia sativa*, and *Hedysarum coronarium*. In the Solanaceae there are varieties at least of *Capsicum annuum*, *Solanum melongena*, *Solanum tuberosum*, *Hyoscyamus niger*, and certain species of Nicotiana which are apparently immune to attack. *Benincasa cerifera* is the only species of the cucurbit family tested, which appears to be entirely immune from infection by *Thielavia basicola* when grown in infected soil. The common agricultural plants upon which infection could not be obtained were principally the

cereals—wheat (*Triticum* spp.), oats (*Avena sativa*), barley (*Hordeum* spp.), and rye (*Secale cereale*)—corn (*Zea mays*), potatoes (*Solanum tuberosum*), hemp (*Cannabis sativa*), flax (*Linum usitatissimum*), and sweet potatoes (*Ipomoea batatas*). With the vegetables the cabbage (*Brassica oleracea*), onion (*Allium cepa*), parsnip (*Pastinaca sativa*), carrot (*Daucus carota*), beet (*Beta vulgaris*), lettuce (*Lactuca sativa*), eggplant (*Solanum melongena*), and peppers (*Capsicum annuum*) appeared to be free from attack by the fungus; and of the fruits no infection could be obtained on strawberries (*Fragaria* spp.), raspberries (*Rubus* spp.), or blackberries (*Rubus* spp.).

Infection could not be obtained upon the following plants, which were previously recorded by others as being attacked by *T. basicola: Nicotiana rustica, Phaseolus multiflorus, Pastinica sativa, Scorzonera hispanica, Daucus carota, Apium graveolens,* and *Beta vulgaris.* The failure to obtain infection on these hosts may be attributed to one or more of several obscure causes. The writer is inclined to believe, however, that these species which have been mentioned as hosts of *T. basicola* should not be included in the list of host plants. There is a small probability of immune varieties or strains being used in the studies reported here. In an attempt to clear up this point different varieties of some of these species were used, but only negative results in infection were obtained.

Phaseolus multiflorus and *Nicotiana rustica* are included as hosts of *T. basicola* by Kirchner (9). A search through the literature has failed to reveal earlier reports of these species as hosts. It is evident that Kirchner included these species because of their relation to known susceptible species, as no mention of any original observations of his own could be found. This conclusion is further substantiated by the fact that after repeated tests by the writer, these species appear to be immune to attack.

Aderhold obtained very slight infections on *Scorzonera hispanica, Daucus carota, Apium graveolens,* and *Beta vulgaris.* His results were obtained, however, under quite artificial conditions and no doubt resulted from one form or other of injury to the roots. The conclusion was drawn that *T. basicola* was only a very weak parasite. All attempts to infect the above-named species, under the conditions of these experiments, show that these forms are immune or at least extremely resistant to attack by *T. basicola.* Aderhold's conclusion (1, p. 465)—

> Der Verlauf der Erscheinung zeigte, dass der Pilz kein heftiger Parasit ist. Ich schliesse mich auf Grund dieser Versuche der Auffassung Sorauers an, der Zufolge besondere Verhältnisse geboten sein müssen, um ihn zu einen wirklichen Schädiger zu machen—

should, therefore, certainly not be taken to apply to other host plants of *T. basicola.*

Massee (11) includes *Blysmus compressus* as a host of *T. basicola*, apparently on the basis of an early report (10) of *Milowia nivea* on decaying leaves of *B. compressus*. Unless this observation was corroborated by later observation, the writer believes some doubt may be entertained in regard to this species as a host, on account of the point of attack and question of the identity of this fungus with *T. basicola* Zopf. The description and figures in Massee's first article bear only a slight resemblance to *T. basicola*. Unfortunately the writer has been unable to obtain this species of Blysmus for trial in these experiments.

It is also interesting to note that certain species commonly cited as hosts of *T. basicola* are strikingly resistant or practically immune. Chittenden (4–5), Massee (11), and Taubenhaus (21) report *T. basicola* as a serious disease of sweet peas (*Lathyrus odoratus*), though they differ in their opinions as to the symptoms of the disease. Infection of sweet peas in the writer's experiments was obtained only with great difficulty and then only slightly. According to Taubenhaus, the organisms causing this infection of sweet peas and tobacco are interchangeable and no physiological race difference exists in the fungus. It seems plausible, therefore, that more resistant varieties of *L. odoratus* were used in the present trials than were used by Chittenden and Taubenhaus. It may be, therefore, that the sweet-pea disease may be controlled by selection for disease resistance. Taubenhaus also mentioned obtaining a culture of *T. basicola* from parsnip, although he does not include it in his list of hosts. No infection could be obtained upon that vegetable in the present trials.

Aderhold (1) failed to get infection on *Begonia semperflorens*, and it was only after repeated examinations that the writer found the fungus on this host and then only on nematode galls, as reported by Selby for *Begonia rubra*. *Cochlearia armoracia*, *Cyclamen* spp., *Pisum sativum*, *Lupinus albus*, *Catalpa speciosa*, and certain species of orchids were found to be very difficult to infect.

Plants upon which very slight infections were secured in one or two instances but are not included in the new list as hosts are *Lycopersicon esculentum*, *Tropaeolum majus*, *Fagopyrum esculentum*, and *Solanum nigrum*.

As a result of these studies, it is concluded, therefore, that certain members of the following families of plants are likely to be attacked by *Thielavia basicola* under favorable conditions for the development of the fungus: Araliaceae, Bignoniaceae, Compositae, Convolvulaceae, Crucifereae, Cucurbitaceae, Hydrophyllaceae, Leguminosae, Malvaceae, Orchidacae, Oxalidaceae, Papaveraceae, Polemoniaceae, Portulaceae, Primulaceae, Scrophulariaceae, Solanaceae, and Violaceae.

The occurrence of *T. basicola* on the various hosts studied differed principally in two respects: (1) The point of attack by the fungus and (2) the character of the sporulation. The occurrence of *T. basicola* in

certain cases on stems above the surface of the ground has been noted by others (11, 14), but is relatively rarely found. The lesions obtained on *Portulaca oleracea* were almost wholly on the low succulent stems, apparently irrespective of any infection at the base of the stem. Ordinarily, however, infection occurs only on the roots of the host plants or upon the base of the stem just at or below the surface of the soil (Pl. 18). In the case of *Nicotiana* spp., infection is ordinarily found on the secondary roots, although in some species the collar of the plants may be most markedly injured, whereas in the cucurbits the infection is usually on the stem just at or below the surface of the soil (Pl. 18, fig. A). In species of Pisum, Phaseolus, and Lupinus the fungus was ordinarily found first upon the base of the stem or on the primary root (Pl. 18, fig. C). On pansy (*Viola tricolor*) and phlox, where only very slight infections occurred, they were invariably found at the ends of the smallest fibrous roots.

The character of sporulation seemingly differs mostly in the variation of the appearance of the perithecial stage. Many workers have experienced failure or considerable difficulty in locating this stage and have therefore questioned the connection of the perithecia as described by Zopf with the chlamydospores of *T. basicola*. The association of the perithecia upon a large number of the different host plants observed in these tests with the chlamydospore stage of *T. basicola* is fairly convincing as to the correctness of Zopf's conclusions. The perithecia were found to be especially abundant upon *Cucumis maxima*, *Robinia pseudoacacia*, *Cytisus scoporius*, *Nicotiana tabacum*, and to a lesser extent upon a number of other hosts. Although it can not be so stated with certainty, the perithecial stage is apparently never produced in the same way on some host plants, as it is lacking in pure cultures of the fungus. The size, shape, number, and color of chlamydospores produced upon the various hosts differed to some extent. These differences appear to be determined in part by the location of these spore chains. When formed within the host cells, as is common in certain hosts, they were often restricted in their growth and were malformed. In other hosts such as *Cucumis* spp. and *Linaria* spp. they are commonly formed outside of the host cells and are large and uniformly shaped. The color of the chlamydospores varies from a deep blue-black, as on species of Cucumis, to a light brown, as on species of Nicotiana. The conidial spore form is only rarely seen on the living host, although it is produced early and profusely in culture media.

The addition of a large number of new host plants of *T. basicola*, while working with a comparatively small number of species, is taken to mean that the range of host species may perhaps be again doubled in time. Although its pathogenicity was questioned by earlier workers, it is shown that these conclusions were drawn from limited data and that these investigators used species which were either immune or relatively

resistant to attack. The vigorous pathogenicity of this organism on the more susceptible species and varieties can not be questioned. The vigor of the attack on certain susceptible varieties of tobacco at least is as striking as that of any known root disease of plants (Pl. 19). Since the fungus apparently spreads relatively slowly through the cell tissue, however, it seems apparent that soils must be abundantly infected and that a large number of local infections must occur before plants become badly diseased. Considering the wide range of hosts possessed by this fungus, it is possible that large economic losses, which are as yet relatively unknown, may be due to this fungus. In the case of tobacco alone it is certain that an average annual loss amounting to several millions of dollars occurs. Large losses also occur in the culture of violets (*Viola* spp.) and ginseng (*Panax quinquefolium*). Little or nothing is known about the extent of the damage which may be done to the various cultivated leguminous crops under field conditions. It is believed that the fungus will become a serious disease of the peanut (*Arachis hypogaea*) and cotton (*Gossypium* spp.), if it has not already become one.

SUMMARY

(1) *Thielavia basicola* Zopf is a fungus parasite attacking primarily members of the Leguminosae, Solanaceae, and Cucurbitaceae. Other families containing hosts of this fungus are Araliaceae, Bignoniaceae, Compositae, Convulvulaceae, Cruciferae, Hydrophyllaceae, Malvaceae, Orchidaceae, Oxalidaceae, Papaveraceae, Polemoniaceae, Portulaceae, Primulaceae, Scrophulariaceae, and Violaceae.

(2) Infection could not be obtained upon the following species of plants reported by others as hosts of *T. basicola: Phaseolus multiflorus*, *Nicotiana rustica*, *Scorzonera hispanica*, *Daucus carota*, *Apium graveolens*, *Beta vulgaris*, and *Pastinica sativa*.

(3) Thirty-nine species of plants have been reported by earlier investigators as hosts of *T. basicola*. Thirty-two of the thirty-nine reported hosts plants have been grown in soil infected with *T. basicola*, and infection obtained upon twenty-five of these plants. Of the seven upon which negative results in infection were secured, it is believed that all should be excluded from the list of hosts until further corroboratory evidence of infection is obtained. The remaining seven species could not be tested, owing to the difficulty of getting seeds or plants.

(4) Sixty-six new species of plants are added as hosts of *T. basicola*, of which twenty-eight are legumes, twenty are solanaceous plants, seven are cucurbits, and eleven belong to miscellaneous families.

(5) A great difference in the susceptibility of the various species exists; and where earlier workers have been inclined to doubt the parasitism of *T. basicola*, it appears to have been due to the fact that infection experiments were carried on with what are now known to be immune or very resistant plants.

(6) Some differences in point of attack and character of sporulation of *T. basicola* on different hosts have been noted. The common occurrence of the perithecial stage in close association with the chlamydospores of a number on different hosts is taken as good indirect corroboratory evidence of Zopf's connection of this form with *T. basicola*.

(7) The infection of nearly 100 different species of plants with *T. basicola* from tobacco is further evidence that no specialized races of this fungus appear to exist.

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PLATE 18

Fairly typical diseased spots and lesions caused by *Thielavia basicola* on various host plants. Some of the infected areas are indicated by arrows and braces:

Fig. A.—*Citrullus vulgaris* (citron).

Fig. B.—*Onobrychis viciaefolia* (sainfoin).

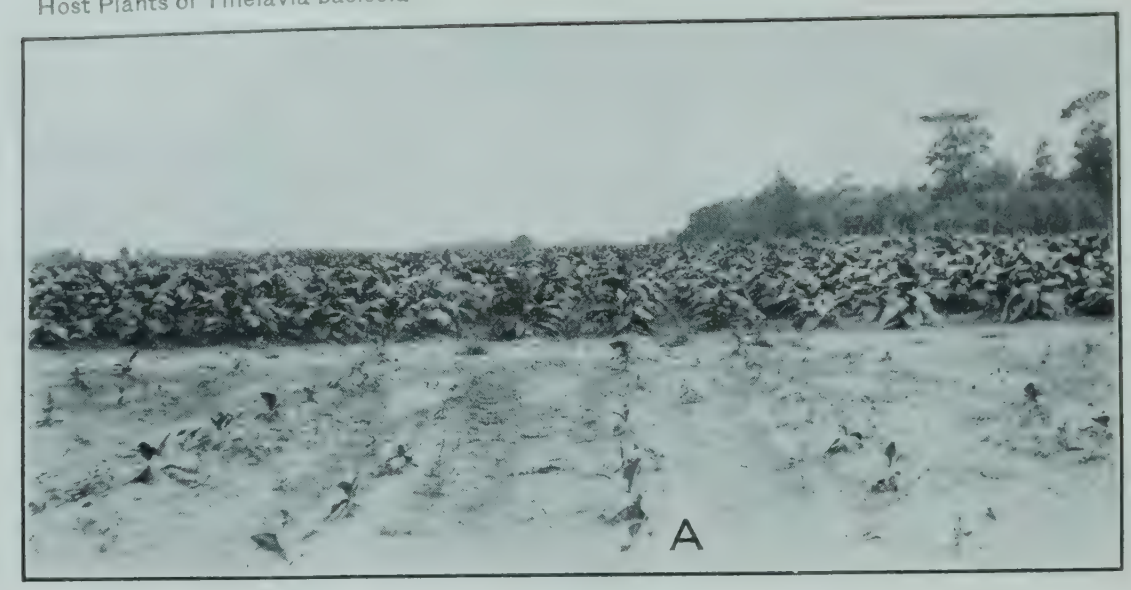
Fig. C.—*Lupinus luteus* (yellow lupine).

Fig. D.—*Arachis hypogaea* (peanut).

Fig. E.—*Robinia pseudoacacia* (black locust).

Fig. F.—*Sclotis chinensis* (wistaria).

A
B
C
D
E
F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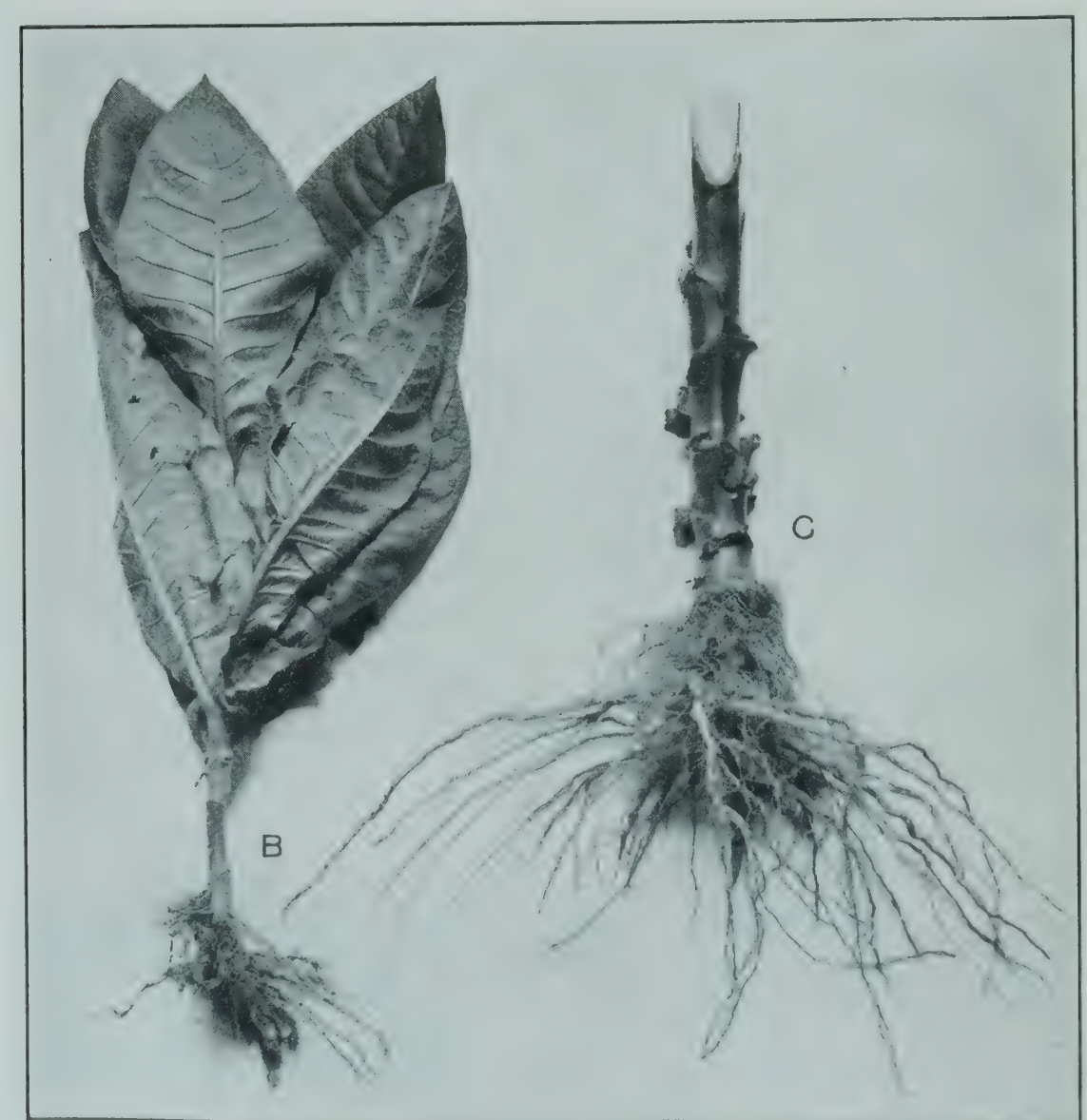
B
C

PLATE 19

Fig. A.—Part of a field infected with *Thielavia basicola* in foreground, with newer soil planted to tobacco in the background, illustrating the marked pathogenic powers of this organism.

Fig. B.—A tobacco plant showing diseased roots from infected soil.

Fig. C.—Healthy roots from uninfected soil of a semiresistant type of tobacco (Connecticut Havana). Figures B and C show the relative growth of plants and amount of root system after equal care in removing roots from the soil.

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CHEMICAL COMPOSITION, DIGESTIBILITY, AND FEEDING VALUE OF VEGETABLE-IVORY MEAL[1]

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INTRODUCTION

Vegetable ivory, or the corozo nut, as it is commonly known in commerce, is the seed or nut of the palmlike plant *Phytelephas macrocarpa*. It is a native of the Latin American countries, being found in great quantities along the banks of the Magdalena, in Colombia, where it is known as "tagua." It is also found in Peru and in the forests of northern Ecuador. In appearance the plant itself is a stemless palm, bearing its fruit in conglomerate heads, often weighing 25 to 30 pounds apiece. These heads are made up of 30 to 50 seeds or nuts, varying in size from half an inch to several inches in diameter. In the earlier stages of growth the seed contains a clear, insipid liquid, which later changes to a sweet, milky paste, and finally hardens into the white horny substance from which it derives its name "vegetable ivory."

Large quantities of the nuts are imported annually by Great Britain and Germany, principally for the manufacture of buttons. The United States uses about 10,000 tons annually, costing $1,500,000 (1, p. 200).[2] Beneath the brown outer coating the dried nut has the appearance of dentine ivory, and can easily be sawed, carved, and turned into all sizes and shapes of buttons, while the texture is such that it readily absorbs dyes and will take a high polish.

In the process of manufacture a considerable portion of the nut is wasted in the form of sawdust, chips, and turnings. In foreign countries this waste has been mixed with other ingredients to be used as a cattle food. German writers state that vegetable-ivory meal has been used

[1] From the Department of Chemistry, Massachusetts Agricultural Experiment Station. Printed with the permission of the Director of the Station.

[2] Reference is made by number to "Literature cited," p. 320.

as an adulterant in the manufacture of so-called concentrated feeds. Some instances are given where as much as 50 per cent of this material has been added.

In the last few years considerable attention has been attracted in this country to the enormous amount of waste material produced by ivory-button factories, and many attempts have been made to discover a practical use for the material aside from fuel.

The material experimented with had the appearance of a medium-fine meal, white in color, though flecked here and there with particles of the brown outer coating of the nut. It was tasteless, odorless, and very hard, being almost gritty to the touch.

CHEMICAL COMPOSITION OF VEGETABLE-IVORY MEAL

Considerable work has been done on the chemistry of vegetable ivory both in this country and in Europe. Tollens (14, p. 113), Fischer and Hirschberger (7), Liebscher (11), and others have worked with ivory meal as a source of mannose and have otherwise investigated the material.

A considerable amount of corroborative chemical investigation of vegetable ivory has been undertaken and an attempt made to determine quantitatively the mannose present. The results are presented with as little detail as possible, the fodder analyses being given in Table I.

TABLE I.—*Fodder analyses of vegetable ivory*

Constituent.	Maximum.	Minimum.	Average.[a]	German analyses for comparison.
Moisture	12.64	6.13	11.39	18.30–13.20
Ash	2.30	.80	1.08	1.30– 1.10
Protein	5.26	3.94	4.63	4.60– 4.00
Fat	1.18	.60	.92	1.10– 0.80
Fiber	7.75	6.13	6.89	79.80–75.80
Nitrogen-free extract	77.56	74.17	75.09	

[a] Average of nine samples.

At a glance Table I shows not only the variations met with in different samples but that by far the greater part of the material is carbohydrate in nature. The protein rarely exceeds 5 per cent and was found to contain about one-third of its nitrogen in the amido form. The fat or ether extract had the appearance of a heavy light-colored oil and possessed a pleasant nutty odor.

The fiber in all cases was fairly uniform in amount, being about 7 per cent of the dry matter. It was noticed while making the determinations that the vegetable ivory acted as an indicator, the change from light buff with acid to a deep wine color with alkali being quite abrupt. Both the residue from the fiber determinations and the original material were

tested for the presence of lignin, but neither phloroglucinol nor anilin sulphate produced any color reaction whatever. As three-fourths of the vegetable ivory was found to be nitrogen-free extract, it was to this portion that the most attention was given.

It has long been known that the greater part of the carbohydrate material consists of mannose, or, more accurately speaking, mannan, its anhydrid condensation product.

The isolation of mannose was carried out practically as described by Fischer and Hirschberger (7). One hundred gm. of vegetable-ivory meal were digested on the water bath with reflux condenser for six hours with 200 c. c. of 6 per cent hydrochloric acid. The liquid was then filtered off, the filtrate and washings neutralized with sodium hydroxid, and shaken out several times with carbon black. After filtration, phenylhydrazin (dissolved in acetic acid) was added at the rate of 0.3 gm. for every gram of ivory meal used. The mannose phenylhydrazone separated out on standing for 24 hours in the cold as a heavy, fine-grained, buff-colored precipitate. This was washed with cold water and dried in a vacuum at room temperature. Particles of this impure hydrazone when placed in a capillary tube and heated slowly in a sulphuric-acid bath melted at 183° C.

A portion of the precipitate was purified by boiling for a long time with a large volume of 95 per cent alcohol, filtering, and again boiling with fresh alcohol until at the end of two days an almost snow-white hydrazone resulted. This melted at 196° C., demonstrating the existence of mannose or its polymer mannan in vegetable ivory.

To liberate mannose from its phenylhydrazone, a portion of the latter was digested with benzaldehyde and alcohol until crystals of benzaldehyde hydrazone formed. The mannose containing filtrate from these, after clarifying and evaporating to a sirup, was treated with absolute alcohol and set aside to crystallize. The mannose crystals obtained had a melting point of 132° C.

Pentosans were determined by the hydrochloric-acid distillation method and precipitation with phloroglucinol. The average of three determinations was 2.43 per cent of the dry matter.

Repeated attempts to produce mucic acid by oxidation with nitric acid proved futile. The exact method for the detection and determination of galactan was carried out always with negative results.

Microscopic examination with iodin failed to give the slightest evidence of starch, either in the white fleshy part of the nut or in the brown outer coating.

Dextrose (or dextran) was shown to be absent in vegetable ivory by its inability to form saccharic acid. As a check on the method used, a sample of pure glucose was treated exactly as was the ivory meal. No difficulty was experienced in obtaining the saccharic acid from the check.

It was found by boiling a little of the vegetable-ivory meal in water, filtering out the insoluble portion, and then adding the clear filtrate to a large volume of strong alcohol that a precipitate would form after standing for some time. Since this process is similar to that employed in separating pectin (plant mucilage) from fruits, it was first supposed to be the same product. The amount present was found to be 2.78 per cent on a dry-matter basis.

According to the best authorities, pectin, supposed by many to be an oxygen or acid derivative of cellulose, is readily oxidized to mucic acid by proper treatment with nitric acid. The product from vegetable-ivory meal when so treated produced no mucic acid. That it could not be of pentose character was demonstrated by making determinations on the filtered precipitate. Not the slightest trace of phloroglucid formed, showing the absence of five carbon sugars.

From various authorities and from actual observation, pectin derived from fruit is known to reduce Fehling's solution. Indications of such reduction were not noted in the case of this precipitate.

On the supposition that it might be of a nitrogenous nature, a nitrogen determination was made with negative results.

Considering these results, it would seem that the alcoholic precipitate from vegetable-ivory meal is distinctly different from the so-called "plant mucilage."

In attempting the determination of the sugars present in vegetable-ivory meal by Fehling's gravimetric method, many difficulties were encountered. However, a brief summary of the results obtained seems worthy of note. Water extracts of the material without inversion gave about 0.5 per cent of reducing material. The same solution after hydrolysis with hydrochloric acid at 20° C. for 24 hours gave an average of 2 per cent of reducing material. From this it was evident that the mannose, or, more properly speaking, the mannan, existed as a hemicellulose, since otherwise the total sugars would have been in an enormous excess of 2 per cent. Consequently the hydrolysis was made more drastic by boiling, and it was found that with an increase in the length of the boiling period the percentages of sugars increased (Table II).

TABLE II.—*Relation of the length of the boiling period to the percentage of sugars in vegetable-ivory meal*

Hours boiled.	Percentage of sugar (as dextrose).
2½	47.40
4	65.00
5	73.40
6	73.40
7	73.40

It was noticed that five hours' boiling in an acid solution was necessary to hydrolyze completely the mannose and other reducing materials and that more than five hours' boiling produced no increased percentage.

The percentages were calculated not as mannose but as dextrose, since no table for the determination of mannose by Fehling's method has been found.

The pentosans present no doubt had a somewhat different reducing capacity than the mannan. However, when appropriate allowance had been made for moisture and pectin (previously determined), the total carbohydrates estimated in this fashion approached to within less than 1 per cent of the amount estimated as nitrogen-free extract in the original fodder analysis. It is evident, therefore, that the so-called nitrogen-free extract, comprising fully 75 per cent of the vegetable-ivory meal, was composed principally of mannan with small amounts of pentosans and of a substance insoluble in alcohol but not identical with the pectin substances as usually found in plants.

CALORIFIC VALUE OF VEGETABLE-IVORY MEAL

To determine the calorific value of this substance a number of bomb-calorimeter determinations were made, the average of which is given in Table III, together with representative figures for other common substances used as food.

TABLE III.—*Comparative calorific values of vegetable-ivory meal, corn meal, sugar, and cornstarch*

Material.	Small calories, per gram.	Large calories, per pound.
Vegetable-ivory meal	3,785	1,717
Corn meal (9, p. 405, 420)[1]	3,549	1,610
Sugar (guaranteed)	3,958	1,753
Cornstarch (9, p. 405, 420)	3,692	1,675

[1] H. P. Armsby (2, p. 13) reports corn meal as having a chemical energy of 170.9 therms per 100 pounds, the equivalent of 3,766 small calories per gram, or 1,709 large calories per pound.

In button factories, where the largest amount of ivory waste, or meal, is produced, the material is used under the boilers as fuel, and it has been authoritatively stated[1] that it produces about half as much heat as coal. It is interesting to note how accurately this statement is borne out scientifically. The average of 20 samples of soft coal recently analyzed at this station was 14,074 B. T. U., which, expressed in large calories per pound, equals 3,546. This figure is approximately twice that of the vegetable-ivory meal, 1,717.

[1] Courtesy of Mr. C. J. Spill, Superintendent of the United Button Co., Springfield, Mass.

DIGESTION EXPERIMENTS

EXPERIMENT I.—The determination of digestibility was carried out with two sheep in the usual manner (12). The sheep were fed a ration consisting of 500 gm. of English hay, 150 gm. of gluten feed, 200 gm. of finely ground vegetable-ivory meal, and 10 gm. of salt with water *ad libitum*. Rations for the entire test were weighed out at the beginning and samples sent to the laboratory and immediately analyzed. The results are given in Table IV.

TABLE IV.—*Percentage composition of feedstuffs used in Experiment I*

Constituent.	Hay.	Gluten feed.	Vegetable-ivory meal.
MOISTURE			
Moisture	11.95	10.07	12.64
Dry matter	88.05	89.93	87.36
Total	100.00	100.00	100.00
DRY MATTER			
Ash	5.81	1.11	1.37
Protein	9.34	26.96	6.02
Fat	2.88	3.94	.67
Fiber	31.70	8.70	7.02
Nitrogen-free extract	50.78	59.29	84.90
Total	100.00	100.00	100.00

The experiment lasted 14 days, 7 of which were preliminary; and during that time no disturbances in digestion were observed. The sheep ate the vegetable-ivory mixture readily.

One-tenth of the daily manure excreted by each sheep was carefully dried and preserved. Later these portions were composited and analyzed, the results being given in Tables V and VI.

TABLE V.—*Quantities of manure and urine excreted and water consumed daily by sheep fed vegetable-ivory meal, gluten feed, and hay*

SHEEP 5

Date.	Manure.			Urine.	
	Total weight.	Weight of the one-tenth preserved.	Air-dry weight.	Weight.[a]	Nitrogen in urine.
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Per cent.*
Nov. 16	711	71. 1	29. 25	2, 151	0. 48
Nov. 17	626	62. 6	28. 20	975	. 83
Nov. 18	684	68. 4	28. 63	1, 397	. 58
Nov. 19	476	47. 6	21. 20	2, 201	. 64
Nov. 20	476	47. 6	20. 45	1, 135	. 68
Nov. 21	536	53. 6	23. 37	1, 459	. 57
Nov. 22	615	61. 5	25. 37	914	. 80
Average	589	58. 9	25. 21	1, 461	. 65

SHEEP 6

Nov. 16	535	53. 5	25. 21	621	1. 73
Nov. 17	527	52. 7	25. 45	665	1. 34
Nov. 18	495	49. 5	23. 21	681	1. 25
Nov. 19	483	48. 3	23. 39	547	1. 42
Nov. 20	545	54. 5	25. 89	643	1. 40
Nov. 21	407	40. 7	19. 93	543	1. 08
Nov. 22	512	51. 2	26. 28	647	1. 31
Average	507	50. 7	24. 19	621	1. 36

[a] The quantity of urine in each case was increased by 100 c. c. of carbolic disinfectant and wash water used at the barn.

TABLE VI.—*Percentage composition of feces of sheep fed vegetable-ivory meal, gluten feed, and hay*

MOISTURE

Constituent.	Sheep 5.	Sheep 6.
Moisture	5. 70	5. 50
Dry matter	94. 30	94. 50
Total	100. 00	100. 00

DRY MATTER

Ash	9. 16	9. 80
Protein	15. 60	14. 70
Fat	3. 96	3. 44
Fiber	25. 96	25. 34
Nitrogen-free extract	45. 32	46. 72
Total	100. 00	100. 00

The sheep were weighed on the first two and the last two days of the digestion period and the average taken to determine gain or loss in body weight (Table VII).

TABLE VII.—*Gain or loss in weight (pounds) by sheep fed vegetable-ivory meal, gluten feed, and hay*

Sheep No.	Weight at beginning.	Weight at end.	Gain.	Loss.
5	139. 25	137. 25	.....	2. 00
6	160. 13	163. 83	3. 70	.....

As a supplementary check on the metabolism of the sheep, the urine was collected, weighed, and sampled daily, and the nitrogen determined. A study of Table VIII shows that sheep 5 excreted more nitrogen than was supplied in its food. This sheep lost in weight. Sheep 6, however, gained in body weight, and it will be noted that less nitrogen was given off than was consumed.

TABLE VIII.—*Nitrogen balance of sheep fed vegetable-ivory meal, gluten feed, and hay*

[Estimated in grams of protein.]

Sheep No.	Consumed.	Excreted.	Gain.	Loss.
5	647. 92	659. 07	.....	11. 15
6	647. 92	606. 69	41. 23	.....

By applying the analyses in Table IV to the total rations fed, the total amounts of dry matter and food constituents are obtained. From these the amounts of the several constituents of the manure (calculated by the use of Table VI) are subtracted. The remainder is the quantity of hay, gluten, and vegetable-ivory meal digested. By subtracting from this the amount of hay and gluten digested [1] the amount and percentage of the vegetable ivory digested is obtained (Table IX).

[1] Obtained by applying the digestion coefficients of hay and gluten alone to the quantity fed.

TABLE IX.—*Daily consumption and excretion (in grams) and the digestion coefficients of sheep fed vegetable-ivory meal, gluten feed, and hay*

SHEEP 5

Item.	Dry matter.	Protein.	Fat.	Fiber.	Ash.	Nitrogen-free extract.
550 gm. of English hay	484. 28	45. 67	11. 04	153. 52	28. 14	245. 91
150 gm. of gluten feed	134. 90	36. 37	5. 32	11. 74	1. 50	79. 97
200 gm. of vegetable ivory	174. 72	10. 52	1. 21	12. 27	2. 39	148. 33
Amount consumed	793. 90	92. 56	17. 57	177. 53	32. 03	474. 21
Minus 252.10 gm. of manure	237. 73	37. 09	9. 41	61. 71	21. 78	107. 74
Hay, gluten, and vegetable ivory digested	556. 17	55. 47	8. 16	115. 82	10. 25	366. 47
Minus hay and gluten digested	408. 66	55. 79	9. 16	109. 07	9. 19	228. 12
Vegetable ivory digested	147. 51	.....	.....	6. 75	1. 06	138. 35
Percentage digested	84. 43	.....	.....	55. 01	44. 35	93. 27

SHEEP 6

Amount consumed (as per sheep 5)	793. 90	92. 56	17. 57	177. 53	32. 03	474. 21
Minus 241.94 gm. of manure	228. 63	33. 61	7. 86	57. 93	22. 41	106. 82
Hay, gluten, and vegetable ivory digested	565. 27	58. 95	9. 71	119. 60	9. 62	267. 39
Minus hay and gluten digested	408. 66	55. 79	9. 16	109. 07	9. 19	228. 12
Vegetable ivory digested	156. 61	3. 16	. 55	10. 53	. 43	139. 27
Percentage digested	89. 63	30. 04	45. 45	85. 82	17. 99	93. 89
Average percentage for both sheep	87. 03	[a] 30. 04	[a] 45. 45	70. 42	31. 17	93. 58
Percentage digestibility of English hay and gluten as previously determined [b]	66	68	56	66	31	70

[a] One sheep only.

[b] Obtained from previous digestion experiments similar to the one under discussion, in which a part of the same lot of hay and gluten feed was used.

Table IX shows that the two digestion trials for nitrogen-free extract agree very closely. It is to be noted further that this extract matter constituted about 85 per cent of the total dry matter of the vegetable-ivory meal and that it had a digestibility of 94 per cent. In the case of the fat and ash the results are uncertain, but this is not surprising because so very little of these two ingredients is present. The digestibility of the fiber is not very satisfactory, and the same may be said of the protein. The percentages of these two ingredients, however, in the vegetable-ivory meal are relatively small.

EXPERIMENT II.—In another similar experiment conducted with three sheep and a different sample of vegetable-ivory meal the following digestion coefficients were obtained. With them are compared the results of Experiment I as well as the average coefficients for corn meal (Table X).

TABLE X.—*Comparison of the digestion coefficients obtained in Experiments I and II*

Experiment No.	Feed.	Number of sheep.	Dry matter.	Protein.	Fat.	Fiber.	Nitrogen-free extract.
I	Vegetable ivory	2	87	30	[a] 45	70	94
II	do	3	81	[b] 41	56	73	89
Average			84	36	51	72	92
Corn meal (13, p. 295)			88	67	90	.....	92

[a] One sheep only. [b] Different sheep showed variable results.

Of these figures the first and last two columns demand the most attention. Corn meal contains nearly as much nitrogen-free extract as the vegetable ivory, and it would appear that the percentage digestibility of this ingredient in each feed is approximately the same.

Applying the average coefficients to the composition of the dry matter of the vegetable-ivory meal and the average coefficients for corn meal to the dry matter contained in the latter[1] and multiplying by 2,000, one obtains the following amounts of digestible matter in 1 ton of each of the two feeds (Table XI).

TABLE XI.—*Digestible nutrients (in pounds) in vegetable-ivory meal and corn meal per ton*

Feed.	Protein.	Fat.	Fiber.	Nitrogen-free extract.	Total.
Vegetable ivory	42.34	6.83	101.08	1,582.20	1,732.45
Corn meal	147.52	78.84	.....	1,486.40	1,712.76

On the basis of total digestible organic matter the results indicate that the vegetable-ivory meal is equal in feeding value to corn meal. Kellner (10) and Armsby and Fries (3, 4, 5, 6) have shown, however, that it is not possible to estimate with accuracy, by means of digestion experiments, the relative value of different feedstuffs. In view of the excess of fiber in the vegetable-ivory meal over that of the corn meal (7 per cent in ivory meal *v.* 2 per cent in corn meal), of the tough horny nature of the ivory nut, of the uncertainty of the nutritive value of the mannan as compared with starch, and of the unknown influence of the two feedstuffs

[1] The average composition of corn meal on a dry-matter basis in Lindsey's compilations (12) is protein 11.01 per cent, fat 4.38 per cent, fiber 2.25 per cent, and nitrogen-free extract 80.79 per cent.

on metabolism, one is justified in assuming that the vegetable-ivory meal can not have as high a nutritive effect as has the corn meal.

EXPERIMENT III.—As another means of determining how completely vegetable-ivory meal was digested, Experiment III was undertaken, feeding the same amounts of the several feeds as in Experiment I. The basal ration consisted of hay and gluten feed, and the ration proper of the same feeds in like quantities, plus 200 gm. of vegetable-ivory meal. Each ration was fed for 14 consecutive days, the feces being collected for the last 7 days in each period, and aliquots preserved. In this experiment the hay, gluten feed, and vegetable-ivory meal ration preceded the basal ration of hay and gluten feed. The feces were tested for total sugar after acid hydrolysis, to note whether the percentage of sugar was higher in the ivory-meal period than in the period without the meal. It is understood that little or no sugar should appear as such in normal feces, and the relatively large amounts which are reported below are accounted for as a result of the hydrolysis of pentosans and other hemicelluloses, largely from the hay. It was necessary to hydrolyze with strong acid and boiling in order to include completely the sugar of the vegetable ivory, if any, which might have passed through the animal unchanged.

On a dry-matter basis it was found that the average carbohydrate content, estimated as dextrose, for the feces of the hay, gluten, and ivory-meal period was 25.46 per cent and that for the hay and gluten period was 24.68 per cent. In other words, the total amount of carbohydrates, so called, found in the feces when vegetable ivory had been included in the ration was only 0.78 per cent more than was found when it had not been included. This is relatively such a small amount that it seems safe to conclude that very little, if any, of the carbohydrate of the vegetable ivory escaped undigested. The mannan therefore appears to have been quite thoroughly hydrolyzed and assimilated by the sheep.

FEEDING EXPERIMENTS

EXPERIMENT I.—During March, April, and May, 1914, an experiment to compare the relative feeding value of vegetable-ivory meal and corn meal was carried out and may be described as follows:

Three pairs of cows were fed for periods of five weeks each, exclusive of preliminary periods of 10 days, on basal rations consisting daily for each cow of substantially 2.5 pounds of wheat bran, 2.5 pounds of cottonseed meal, and what hay the animals would eat clean (about 20 pounds). Either 3 pounds of vegetable-ivory meal or 3 pounds of corn meal were fed in addition. The experiment was conducted on the reversal plan—that is, one cow of each pair was fed the basal ration plus the vegetable ivory for five weeks, while the other received corn meal; then the ration was reversed for five weeks (Table XII).

TABLE XII.—*Average daily ration (in pounds) consumed per cow in Feeding Experiment I*

Character of ration.	Hay.	Bran.	Cottonseed meal.	Corn meal.	Vegetable-ivory meal.
Corn meal	20. 58	2. 36	2. 28	3. 36	.....
Vegetable-ivory meal	20. 46	2. 36	2. 28	1	3

Daily samples of the grain and ivory meal were taken and preserved in glass-stoppered bottles. These were brought to the laboratory for analysis at the end of each half of the trial. The hay was sampled three times during each half and determinations of moisture made and aliquots preserved for analysis.

Milk samples were taken for five consecutive days in the first, third, and fifth weeks of each half of the test and analyzed for fat and total solids.

The cows were weighed on two consecutive days at the beginning and end of each half of the experiment.

The cows averaged 0.12 of a pound more hay daily while on the corn-meal ration. The 1 pound of corn meal fed during the vegetable-ivory period applied only to two of the cows for periods of 35 days each.

From the analyses of the materials fed, their digestibility coefficients, and the amounts daily consumed, the quantity of digestible organic nutrients received daily per cow was estimated, the results being given in Table XIII.

TABLE XIII.—*Quantity of digestible organic nutrients in the average daily ration of cows in Feeding Experiment I*

Character of ration.	Protein.	Fat.	Fiber.	Nitrogen-free extract.	Total.	Nutritive ratio.
Corn meal	2. 03	0. 53	4. 07	8. 86	15. 47	1 : 6. 9
Vegetable-ivory meal	1. 88	. 43	4. 29	9. 17	15. 77	1 : 7. 6

It will be noticed that the total digestible carbohydrates were slightly greater for the vegetable-ivory ration than for the corn-meal ration. On the other hand, protein and fat show a favorable balance for the corn-meal ration.

At the completion of the experiment a distinct gain in herd weight was noticed when the corn-meal ration was fed over that of the vegetable-ivory ration (Table XIV).

TABLE XIV.—*Gain or loss (in pounds) in herd weight in Feeding Experiment I*

Character of ration.	Gain.	Loss.	Average per cow.	
			Gain.	Loss.
Corn meal	94	.....	15. 6	.....
Vegetable-ivory meal	6	.....	1. 0	.....

Table XV records the total yield of milk and milk ingredients per cow for each ration, as well as the total yield of the herd.

TABLE XV.—*Total yield of milk and milk ingredients from different rations in Feeding Experiment I*

CORN-MEAL RATION

Name of cow.	Time.	Milk.	Solids.		Fat.		Butter.[a]
	Weeks.	*Pounds.*	*Per cent.*	*Pounds.*	*Per cent.*	*Pounds.*	*Pounds.*
Samantha II..	5	1,184.6	12.57	148.9	4.03	47.7	54.9
Fancy III....	5	1,131.8	12.90	146.0	4.33	49.0	57.2
Betty.....	5	761.6	13.76	104.8	4.77	36.3	42.3
Betty II.....	5	933.4	13.76	128.8	4.75	44.3	51.7
Amy.....	4	673.8	13.45	90.6	4.68	31.5	36.7
Amy II.....	4	558.3	14.75	82.3	5.45	30.4	35.5
Total...	28	5,243.5	[b] 13.37	701.0	[c] 4.56	239.2	278.3

VEGETABLE-IVORY-MEAL RATION

Samantha II..	5	1,200.6	12.70	652.5	4.13	49.6	57.9
Fancy III....	5	1,001.6	13.01	130.3	4.53	45.4	53.0
Betty.....	5	805.0	13.80	111.1	4.93	39.7	46.3
Betty II.....	5	897.6	13.61	122.2	4.49	40.3	47.0
Amy.....	4	620.7	13.26	82.3	4.74	29.4	34.8
Amy II.....	4	547.2	15.15	82.9	5.79	31.7	37.0
Total...	28	5,072.7	[b] 13.43	681.3	[c] 4.65	236.1	276.2

[a] Butter equals fat plus one-sixth.
[b] Averages obtained by dividing the total weight of solids by the total weight of milk.
[c] Averages obtained by dividing the total weight of fat by the total weight of milk.

One hundred and seventy pounds more milk were produced by the corn-meal ration than by the vegetable-ivory-meal ration. This excess is not pronounced; and while it is possible that the difference may be within the limit of experimental error when taken together with the fact that the corn-meal ration increased the live weight of the cows, it indicates at least that the corn meal was somewhat superior to the vegetable-ivory meal as a source of nutrition.

TABLE XVI.—*Average percentage composition of milk of the herd in Experiment I on each ration*

Character of ration.	Total solids.	Fat.	Solids not fat.
Corn meal.....	13.37	4.56	8.81
Vegetable-ivory meal.....	13.43	4.65	8.74

The very concisely stated data of Table XVI of this experiment indicate that vegetable-ivory meal possesses a distinct feeding value and that, while somewhat inferior to corn meal, the difference is not marked.

As greater difficulty was met with in hydrolizing the carbohydrate of the vegetable ivory than the carbohydrate of other feeding materials, it

would seem reasonable to suppose that the same relation would hold true in the digestive processes of animals. This being the case, more of the total energy of the material would be used up in digestion and assimilation, which otherwise might be used for milk production.

EXPERIMENT II.—A short experiment with three cows, supplementary to Experiment I, was carried out during January and February of 1915. A basal ration somewhat below what the cows needed for maintenance and milk was fed for two weeks. Then the cows were given 3 pounds of vegetable-ivory meal in addition for two weeks. During the fifth and sixth weeks the cows were again given only the basal ration. The object was to see whether the animals would show any increase in weight or milk production in response to the addition of the ivory meal.

TABLE XVII.—*Average daily ration (in pounds) consumed per cow in Feeding Experiment II*

Character of ration.	Hay.	Wheat bran.	Cottonseed meal.	Hominy.	Vegetable-ivory meal.
Basal	18. 67	2. 34	2	1	.....
Vegetable-ivory meal	18. 67	2. 34	2	1	3

The quantity and quality of the milk was determined for the second week of each section of the experiment. Samples were taken in the usual way and the animals were weighed on two consecutive days at the beginning and end of each second week.

Little can be said about body weight, as the periods were of too short duration.

TABLE XVIII.—*Yield of milk and milk ingredients from different rations in Feeding Experiment II*

BASAL RATION, JANUARY 20–26

Name of cow.	Milk.	Fat.		Solids.	
	Pounds.	*Per cent.*	*Pounds.*	*Per cent.*	*Pounds.*
Amy	196. 5	5. 50	10. 81	13. 77	27. 06
Betty III	137. 6	4. 70	6. 47	13. 23	18. 20
Red III	121. 9	5. 38	6. 56	13. 86	16. 90
Total	456. 0	[a] 5. 22	23. 84	[a] 13. 63	62. 16

VEGETABLE-IVORY-MEAL RATION, FEBRUARY 3–9

Amy	199. 2	4. 90	9. 76	13. 14	26. 17
Betty III	158. 0	4. 58	7. 24	13. 25	20. 94
Red III	125. 1	5. 28	6. 61	13. 71	17. 15
Total	482. 3	[a] 4. 90	23. 61	[a] 13. 30	64. 26

BASAL RATION, FEBRUARY 21–27

Amy	190. 3	4. 80	9. 13	13. 12	24. 97
Betty III	146. 4	4. 60	6. 73	13. 31	19. 49
Red III	125. 4	5. 38	6. 75	13. 93	17. 47
Total	462. 1	[a] 4. 89	22. 61	[a] 13. 21	61. 93

[a] Average obtained by dividing the total number of pounds of fat or solids by the total number of pounds of milk.

Though of short duration, the experiment shows the favorable effects of the addition of 3 pounds per cow daily of the ivory meal to the basal ration. This addition increased the milk flow 5.7 per cent, and its removal caused a decrease of 4.2 per cent.

Another experiment in which a basal ration with and without vegetable-ivory meal was fed three cows was carried on for a period of 81 days. The addition of the meal was followed by an increase in milk yield and its removal resulted in a milk shrinkage. This trial, together with the one just described, shows that the ivory meal possesses a distinct nutritive value.

EXPERIMENT III.—In Experiment I a definite quantity of vegetable-ivory meal was compared with an equal quantity of corn meal. In order to demonstrate more fully the effect of the vegetable ivory, a herd of six cows was put on a basal ration of hay, bran, cottonseed meal, and hominy meal for four weeks, exclusive of a preliminary period of 10 days, during which time three received in addition a quantity of the ivory meal. After this period, which will be known as the first half of the experiment, conditions were reversed, and during the four weeks of the second half the first three cows went without the vegetable-ivory meal while the others received it.

Each cow was weighed before watering and feeding on two consecutive days at the beginning and end of each half of the trial. Samples of hay were taken at the beginning, middle, and end of each half, while the grains were sampled daily. The milk of each cow was sampled in the usual manner on the first, third, and fourth week of each half. The results are given in Tables XIX and XX.

TABLE XIX.—*Average daily ration (in pounds) consumed per cow in Feeding Experiment III*

Character of ration.	Hay.	Wheat bran.	Cottonseed meal.	Hominy.	Vegetable-ivory meal.
Basal plus vegetable-ivory meal...	18. 32	2. 30	2. 22	1. 57	2. 79
Basal minus vegetable-ivory meal.	18. 31	2. 31	2. 23	1. 57	.....

TABLE XX.—*Quantity of digestible organic nutrients in the average daily ration of Feeding Experiment III*

Character of ration.	Protein.	Fat.	Fiber.	Nitrogen-free extract.	Total.	Nutritive ratio.
Basal plus vegetable-ivory meal.....	2. 50	0. 61	3. 04	10. 37	16. 52	1:5. 90
Basal minus vegetable-ivory meal.....	2. 11	. 60	2. 92	8. 36	13. 99	1:5. 97

In order to avoid excessive feeding, a basal ration was fed which was rather below what each animal needed for maintenance and normal milk production. This was calculated on the basis of the writers' knowledge of the individual animal and with the aid of Haecker's standards (8). The addition of the vegetable-ivory meal to this basal ration should therefore prove its distinctive feeding value.

In Table XXI will be found the total yield of milk, fat, and solids produced by each ration.

TABLE XXI.—*Total yield of milk and milk ingredients in Feeding Experiment III*

BASAL RATION PLUS VEGETABLE-IVORY MEAL.

Name of cow.	Milk.	Solids.		Fat.		Butter.[a]
	Pounds.	*Per cent.*	*Pounds.*	*Per cent.*	*Pounds.*	*Pounds.*
Fancy III	1,057.2	13.00	137.43	4.67	49.37	57.6
Betty III	599.0	13.55	81.16	4.72	28.27	33.0
Ida II	608.4	14.38	87.49	5.33	32.43	37.8
Betty	676.0	12.96	87.61	4.39	29.68	34.6
Red III	312.5	15.12	47.25	5.29	16.53	18.3
Amy	705.9	13.27	93.67	4.78	33.74	39.4
Total	3,959.0	[b] 13.50	534.61	[b] 4.79	190.02	220.7

BASAL RATION MINUS VEGETABLE-IVORY MEAL.

Fancy III	970.1	12.52	113.56	4.26	41.33	48.2
Betty III	477.1	12.95	61.78	4.49	21.42	25.0
Ida II	531.7	12.95	68.86	5.16	27.44	32.0
Betty	706.7	13.16	93.00	4.62	32.65	38.1
Red III	433.2	14.38	62.29	5.25	22.74	26.5
Amy	707.8	13.14	93.00	4.84	34.26	40.0
Total	3,826.6	[a] 12.87	492.49	[a] 4.70	179.84	209.8

[a] Butter equals fat plus one-sixth.

[b] Averages obtained by dividing the total weight of fat or solids by the total weight of milk.

It will be noticed here that the ivory-meal ration produced 132.4 pounds more milk than the other ration, an increase of 3.46 per cent. Inasmuch as the total feed consumed was identical in each ration with the exception of 470 pounds of the vegetable-ivory meal, one may conclude in the case of this particular experiment that the 132.4 pounds of milk were produced by the 470 pounds of ivory meal, or that it required 3.56 pounds to produce 1 pound of milk.

If rather less of the basal ration had been fed and more of the vegetable-ivory meal, it is probable that the effect of the latter would have been more pronounced.

EXPERIMENT IV.—Another feeding experiment comparing vegetable-ivory meal and corn meal was carried out during January, February, and March, 1916, with eight cows. In this case, as in Experiment I,

the reversal method was employed, each period continuing five weeks, in addition to the preliminary period. Hay, bran, and cottonseed meal composed the basal ration, to which were added like amounts of dry matter in the form of corn meal or ivory meal (Tables XXII and XXIII). Only a summary is here presented.

All the customary precautions were taken to make the experiment as accurate and representative as possible. The milking was done at the same time each morning and evening, and the animals were weighed at regular intervals. All feed and milk samples were taken in the usual manner.

TABLE XXII.—*Average daily ration (in pounds) consumed per cow in Feeding Experiment IV*

Character of ration.	Hay.	Bran.	Cottonseed meal.	Corn meal.	Vegetable-ivory meal.
Vegetable-ivory meal	18. 5	2. 38	2. 19	.....	3. 75
Corn meal	18. 5	2. 38	2. 19	4. 01	.....

TABLE XXIII.—*Quantity of digestible organic nutrients in the average daily ration in Feeding Experiment IV*

Character of ration.	Protein.	Fat.	Fiber.	Nitrogen-free extract.	Total.	Nutritive ratio.
Vegetable-ivory meal	1. 88	0. 44	3. 58	9. 06	14. 96	1:7. 24
Corn meal	2. 07	. 55	3. 38	9. 01	15. 01	1:6. 57

The calculations indicate that the herd consumed substantially like amounts of digestible nutrients in each of the two rations. One would expect a like effect on body weight and milk production. As in Experiment III, care was taken to feed less digestible nutrients than was required for maintenance and milk yield according to the Haecker standard, in order to secure the maximum effect of each ration. The results indicate that this object was in a measure at least achieved. The herds uffered a loss of weight on each ration, the loss being the greater on the one containing vegetable-ivory meal, 95 pounds, as against 38 pounds for the corn-meal ration.

TABLE XXIV.—*Total yield of milk and milk ingredients in Feeding Experiment IV*

VEGETABLE-IVORY-MEAL RATION

Name of cow.	Total milk.	Solids.		Fat.	
		Average percentage.	Pounds.	Average percentage.	Pounds.
	Pounds.				
Amy	852. 1	13. 60	115. 89	5. 14	43. 80
Betty III	798. 2	13. 30	106. 16	4. 76	37. 99
Cecile II	658. 2	14. 76	97. 15	5. 58	36. 73
White	676. 0	13. 54	91. 53	5. 19	35. 08
Betty II	775. 8	13. 75	106. 67	4. 94	38. 32
Red III	576. 1	13. 69	78. 87	5. 33	30. 71
Red IV	723. 5	13. 29	96. 15	4. 60	33. 28
Samantha II	1, 343. 4	12. 64	169. 81	4. 46	59. 92
Total	6, 403. 3	[a] 13. 47	862. 23	[a] 4. 93	315. 83

CORN-MEAL RATION

Amy	1, 001. 0	13. 23	132. 43	4. 93	49. 25
Betty III	851. 0	12. 83	109. 18	4 43	37. 70
Cecile II	744. 4	14. 28	106. 30	5. 22	38. 86
White	704. 8	14. 10	99. 38	5. 09	35. 87
Betty II	840. 6	13. 83	116. 25	4. 94	41. 53
Red III	599. 5	13. 88	83. 21	5. 39	32. 31
Red IV	736. 0	14. 29	105. 17	5. 20	38. 27
Samantha II	1, 454. 0	12. 65	183. 93	4. 39	63. 83
Total	6, 931. 3	[a] 13. 50	935. 85	[a] 4. 87	337. 72

[a] Averages obtained by dividing the total weight of fat or solids by the total weight of milk.

Table XXIV shows that the corn-meal ration produced an increase of 528 pounds of milk, or approximately 8 per cent over that produced by the ivory-meal ration. The total solids were also increased 73.62 pounds and the milk fat 21.89 pounds. The cows in this experiment were in an earlier stage of lactation than those used in the previous trials, and the results are to be regarded as the most satisfactory. It is evident that while the vegetable-ivory meal possesses a distinct feeding value, a given amount has not the feeding equivalent of the same amount of corn meal. The methods of experimentation with milch cows are not sufficiently sharp to enable one to draw accurate deductions as to the exact relative feeding effect of the two materials.

CONCLUSIONS

(1) Analyses show vegetable ivory to be carbohydrate in nature, containing about 5 per cent of protein and 75 per cent of nitrogen-free extract. Fat and mineral matter are negligible, while crude fiber averages 7 per cent.

(2) Ninety-two and one-half per cent of the nitrogen-free extract is mannan, a polymer of mannose sugar.

(3) Pentosans are present to the extent of 2.5 per cent.

(4) Lignin, galactan, starch, and dextran are not found in vegetable ivory.

(5) A nonnitrogenous "alcoholic precipitate" amounting to about 2.5 per cent is present which is not pentose in nature. It differs from fruit "pectin" in that it does not form mucic acid and does not reduce copper.

(6) By the use of Fehling's solution about 0.5 per cent of water-soluble reducing material and 2 per cent of so-called total sugars are shown to be present after inversion with hydrochloric acid in the cold.

(7) The mannan in vegetable ivory is not entirely hydrolyzed without at least 4½ hours' boiling in an acid solution. The characteristic "acid" color of the solution bleaches out at the completion of hydrolysis.

(8) With continued acid boiling the use of Fehling's solution gives results which, when estimated as dextrose, agree closely with the percentage of nitrogen-free extract minus the percentage "pectin" present. Otherwise stated, practically the entire nitrogen-free extract is accounted for in the form of a hexose sugar or its condensation product, except a small percentage of pentoses and pectin.

(9) The energy equivalent of the material ranks well with other carbohydrate foods, and it possesses a fuel value equal to one-half that of soft coal.

(10) Sheep ate vegetable-ivory meal readily when it was mixed with other grains and digested it very thoroughly. Eighty-four per cent of the dry matter and ninety-two per cent of the nitrogen-free extract were digested.

(11) All the carbohydrates appeared to have been hydrolyzed and absorbed in the digestive tract.

(12) Cows ate the material when mixed with other feed, without evidence of digestive disturbances. They refused to eat it if fed by itself.

(13) When fed as an addition to a basal ration, the increase in milk was sufficient to indicate its positive value as a productive feed.

(14) Though the methods of feeding necessarily followed were not such that exact relative values could be shown, it seems certain that vegetable-ivory meal does not fully equal corn meal for milk production.

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ROSY APPLE APHIS

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INTRODUCTION

The rosy apple aphis is undoubtedly the most injurious leaf-feeding apple aphis. Its attacks not only injure the foliage and deform the growing apple trees (*Malus sylvestris*), but when abundant or unchecked it deforms the fruit, causing the production of "aphis apples," which are unfit for sale. The experiments on which the present paper is based were conducted during the seasons of 1914 and 1915 and the manuscript prepared for publication during the winter of 1915–16. Besides the work recorded in the present paper a study of the embryology was undertaken. This work still remains to be completed, the present paper recording the life history only after the hatching of the egg.[1]

NOMENCLATURE OF ROSY APPLE APHIS

The proper scientific name for the rosy apple aphis has for some years been in doubt. Pergande, as shown by his manuscript notes, always considered it to be *Aphis malifoliae* Fitch. Other American writers usually adopted the view that this name became a synonym of *A. sorbi* Kalt. In studying European specimens and the literature carefully the writers have come to the conclusion that *A. malifoliae* is the only name to apply to the species. Their reasons for this view are pointed out under the different names following.

Aphis malifoliae Fitch, 1854.—Fitch (4, p. 760–761; Repr. p. 56–57)[2] separated our rosy aphis from his *mali* Fab. in connection with his

[1] During the course of the study the writers were assisted by Miss Dorothy Walton and by Mr. James Luckett. Mr. Luckett handled a large number of the experiments during the summer of 1915.

[2] Reference is made by number to "Literature cited," p. 342-343.

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discussion of that species. No complete description was given but enough was recorded to leave no doubt that he had our well-known rosy aphis. The fact that he referred to the winged form as almost entirely black is given by Gillette as sufficient evidence for rejecting the name as not applicable to the insect herein considered. It must be remembered, however, that the insects to which he referred were fall migrants (taken on October 4). These fall migrants are usually much darker than the spring migrants and the writers have reared many at Vienna, Va., which were a uniform jet-black. There seems very good reason, then, for accepting Fitch's name as referring to the species, and since *A. sorbi* and *A. pyri* prove to be distinct, *A. malifoliae* Fitch is the name that must stand for the species.

Aphis pyri Boyer de Fonscolombe, 1841.—This name (1) is the earliest one that has been applied to our rosy aphis. Gillette and Taylor (7, p. 31–32) referred the species to it when they found themselves unable to accept *A. sorbi* Kalt. The original description, however, will not fit our insect, and, although later descriptions given under this name might do so, it will for this reason be impossible to use the name for our common rosy aphis. It may be that Gillette and Taylor based their identification upon the description referred to Boyer's species by Koch (3).

In redescribing what he thought to be the *A. crataegii* of Kaltenbach (2) as well as the *A. pyri* of Boyer, Koch based his descriptions entirely upon apple forms. In this he was describing the forms doubtfully referred to *A. crataegii* by Kaltenbach, but seemingly not the true *A. crataegii* of that author. The description given agrees very closely with our apple insect. It would seem, therefore, that the descriptions of *A. crataegii* Kalt. and *A. pyri* Boyer refer to another insect, but the *A. pyri* Boyer of Koch (3, p. 108–110, fig. 145, 146) is the *A. malifoliae* of Fitch. This insect, however, must be distinguished from the *A. pyri* of Koch (3, p. 60), which is quite a distinct species and has been renamed *A. kochii* by Schouteden.

Aphis crataegii Kaltenbach, 1843.—Kaltenbach (2, p. 66–67) described a species under this name from the white thorn. The description as given does not agree with specimens of the insects under consideration. It seems evident, then, that this name can not be applied to the rosy aphis. In his description, however, Kaltenbach refers to specimens taken on apple which he believed might be the same as those on thorn. It seems to the writers that these forms are the same as ours upon apple and that Kaltenbach was in error in considering them to be the same species as his specimens on thorn. This is indicated by his comparison between the two.

Aphis sorbi Kaltenbach, 1843.—This species (Pl. 20, A) was described (2, p. 70–71) from specimens on Sorbus. This is the name now most uniformly applied to the rosy apple aphis in this country. The first

application of the name here appears to have been made by Sanderson (5, p. 189–191), who went rather fully into the history of the species.

In some characters our rosy apple aphis does not fit the description given by Kaltenbach, and these characters cause the writers to believe that they are dealing with another insect. This belief is strengthened after examination of the European insects. Collections of *A. sorbi* from Sorbus, taken by Mr. J. F. Strauss in Germany, being from the same region and the same host as the original specimens, can with some certainty be considered typical. Moreover, they fit very closely the original description of Kaltenbach.

Although these specimens are in a general way very close indeed to the rosy apple aphis, a careful comparison shows that they represent a distinct species (Pl. 22, C). In the wingless form the cornicles are considerably longer than are those of the rosy apple aphis, and the lateral tubercles are more prominent. The antennæ, too, show a considerable difference, the length of IV, as compared with V, being much less in *A. sorbi* than in the rosy aphis. These characters are well illustrated in Table I.

TABLE I.—*Relative proportions of antennæ and cornicles of wingless forms of Aphis sorbi and A. malifoliae*

Aphis sorbi.					*Aphis malifoliae.*				
Segment III.	Segment IV.	Segment V.	Segment VI.	Cornicle.	Segment III.	Segment IV.	Segment V.	Segment VI.	Cornicle.
22	15	14	8 –24	24	28	20	14	6. 5–27	20
24	17	16	6. 5–22	25	28	19. 5	14	6. 5–28	19
26	17	14	6. 5–25	23	25	21	14	6. 5–27	20
23	16	15	7 –23	22	30	21	15	6. 5–32	20

The winged forms of the two species, while very much alike in general appearance and color characters, can be separated quite easily by measurements of the antennal segments. The specimens of *A. sorbi* show the base of Segment VI considerably longer and the unguis considerably shorter than the same portions of Segment VI of the rosy aphis. The large number of sensoria on the antennæ of the two species cause them to resemble each other very closely.

Measurements of the antennæ of six specimens chosen at random from the two species are given in Table II, together with the number of sensoria on the different segments.

A glance at these data will show a fairly constant difference between the spring migrants of these two forms, and this structural difference is borne out by the writers' experiments. They have been unable to rear the rosy aphis on the host plant of the European *A. sorbi*. The European form they have been unable to test on apples on account of the lack of live material.

TABLE II.—*Relative proportions of antennæ and number of sensoria of winged forms of Aphis sorbi and A. malifoliae*

Aphis sorbi.								*Aphis malifoliae.*							
Segment III.		Segment IV.		Segment V.		Segment VI.		Segment III.		Segment IV.		Segment V.		Segment VI.	
Length.	Sensoria.	Length.	Sensoria.	Length.	Sensoria.	Base.	Unguis.	Length.	Sensoria.	Length.	Sensoria.	Length.	Sensoria.	Base.	Unguis.
34	61	23	23	19	15	10	28	36	60	23	29	15	7	6	30
34	60	24	25	20	10	10	27	38	53	24	27	16	6	6	32
34	53	23	24	20	13	10	29	37	55	24	27	17	10	7	35
34	67	24	24	21	11	9	27	37	63	21	22	15	7	6	35
35	57	22	24	18	9	9	26	36	53	24	27	17	5	7	35
35	64	24	24	20	11	10	27	35	54	22	23	14	4	6	31

Aphis kochii Schouteden, 1903 (Pl. 20, B).—This name was given by Schouteden (6, p. 185) to the species described as *A. pyri* by Koch, since Boyer's species already was called by that name. It was found by Koch curling the leaves of *Pyrus pyraster*. The description given by Koch does not agree with our apple insect, but it does agree with another species occurring on apple in Germany, of which the writers have specimens. Two species were collected from the apple in Germany at the same time, one which seems undoubtedly to be *A. malifoliae* and another which is very similar to it but having short cornicles.

In describing his *A. pyri* Koch says (3, p. 60): "Honigrohrchen sehr kurz, etwas walzenförmig." His figures also show short cornicles which are very unlike those of the rosy aphis, but very much like those of the other species. The writers believe, therefore, that *A. kochii* is quite a distinct species from *A. malifoliae*.[1]

Myzus plantaginis Passerini? (Pl. 24, *A*).—A species of Myzus occurring commonly on the broad-leaved plantain in this country must be distinguished from the rosy apple aphis occurring on plants of the same genus. This is a very simple process where winged forms of the species are present. These have the wing veins bordered with dusky, giving the venation a much heavier appearance than it has in the rosy aphis. The third segment, moreover, of the antennæ has one simple row of sensoria, whereas the same segment of the rosy aphis is crowded with sensoria. When, however, only wingless forms are present, the two species look remarkably alike and there would seem to be almost as much reason for calling the one a species of Myzus as the other. The measurements, too, are very similar, but Segment V of *M. plantaginis* nearly always averages a little longer than Segment IV, whereas in the

[1] Since the account just given was written, Theobald (10, p. 202–210) places *A. malifoliae* Fitch, 1856, as a synonym of *A. kochii* Schouteden, 1903. As shown herein, the two are distinct, but *A. pyri* Boyer of Koch is *A. malifoliae* Fitch.

rosy aphis the reverse is true, Segment IV being usually the longer. Segment III of *M. plantaginis* is also relatively shorter than is the same segment in the rosy apple aphis.

The synonymy of the rosy apple aphis will thus stand as follows:

Aphis malifoliae Fitch

Aphis pyri Boyer of Koch (but not *A. pyri* Boyer nor *A. pyri* Koch).

Aphis sorbi Kaltenbach of recent European and American authors (but not *A. sorbi* Kaltenbach).

Aphis pyri Boyer of Gillette and Taylor (but not *A. pyri* Boyer).

Aphis kochii Schouteden of Theobald (but not *A. kochii* Schouteden).

HISTORY AND DISTRIBUTION OF THE SPECIES

The descriptions given by Koch and Kaltenbach indicate that this species was present in Europe at an early date. It now appears to be well distributed in the apple-growing regions. In America Fitch's description makes its early presence known. It would seem, however, that it was not until about 1900 that the insect assumed the importance of a leading pest. The first reports of its occurrence in injurious numbers came from the Eastern States, but in the few years following it had been observed over a wide area. At present the species occurs over nearly all the apple-growing regions of the country, and in some sections it is very abundant. In some local areas the insect may be not at all common, even though in that general region it is abundant. This seems to be due to the comparative scarcity in some places of its secondary host, and in areas where the insect assumes considerable importance plantain is usually found in great abundance. Although the species occurs abundantly in some of the Northern States, it does not seem as yet to have penetrated very far into Canada. It occurs in Quebec, Ontario, and British Columbia, but nowhere in Canada does it seem to have assumed such importance as in some sections of this country.

METHODS OF STUDY

EXPERIMENTS.—The experiments on which this paper is based were conducted along much the same line as recorded by the writers (11) for the green apple aphis. Eggs were allowed to hatch and the stem mothers and their offspring to grow on young seedlings in pots. The same methods of transfer adopted for the green aphis were employed with this species. As soon as spring migrants were produced, these were transferred to rib-grass plants grown in pots, and covered with lantern globes. In this manner the species was grown throughout the summer, and in the fall the fall migrants and males were returned to the apple. All insects possible were reared to maturity in order that the percentage of winged forms occurring throughout the summer might be ascertained. Winged forms during the summer proved to be rare and this simplified considerably the handling of the insects, since it reduced

the number of lines carried. It is quite possible that lines from stem mothers taken in different localities or possibly different rearing conditions might have resulted in a higher percentage of summer winged forms. All molts and specimens in every generation in each line were mounted for study and these many forms gave an excellent opportunity for determination of variations.

TECHNIQUE.—The technique employed for the study of insects of this species was the same as that recorded in the writers' paper (11) on the green apple aphis.

THE EGG

DESCRIPTION

Size 0.550 by 0.272 mm. The largest were 0.608 mm. long and the shortest 0.480 mm. The width varied from 0.256 to 0.288 mm. The longest eggs were not necessarily the widest. In fact, this was seldom the case.

The newly laid egg is light yellow in color, changing through greenish yellow and yellowish green to black in about four days.

LOCATION ON TREE

The eggs are laid mostly on the small twigs, under buds, or in crevices in the bark. They may, however, be laid on the small branches, or even occasionally on the large branches and trunk. They are seldom laid on the water sprouts, though this also may occur. Usually oviparous females born and reared on water sprouts lay their eggs at the base of such sprouts or on the trunk of the tree.

The small plants used in the experiments were frequently potted with portions of the highest roots exposed. In such cases some oviparous females invariably laid eggs upon these exposed roots.

HATCHING

The eggs of this species commenced hatching, in 1914, about April 8, at the same time that the eggs of *A. pomi* began to hatch, and about 10 days later than *A. avenae*. In 1915 hatching also commenced about April 8. Eggs of *A. pomi* commenced hatching on the same date. At this time the stem mothers of *A. avenae* were in the second instar.

STEM MOTHER

DESCRIPTION

FIRST INSTAR.—Morphological characters. Antennal segments as follows: III, 0.12 to 0.144 mm., average 0.133 mm.; IV, (0.048 plus 0.12 mm.) to (0.064 plus 0.136 mm.). Eyes with few facets. Cornicles short and thick, 0.088 to 0.096 mm., average 0.089 mm.

Color characters: Dark green; appendages and crown black. Insects covered after a short time with a mealy bloom.

SECOND INSTAR.—Morphological characters. Antennal segments as follows: III, 0.112 to 0.114 mm., average 0.128 mm.; IV, 0.072 to 0.096 mm., average 0.081 mm.; V, (0.064 plus 0.136 mm.) to (0.072 plus 0.168 mm.), average (0.069 plus 0.150 mm.).

Segments IV and V quite distinctly imbricated. Eyes with about 35 facets; cornicles 0.12 to 0.128 mm., average 0.123 mm., rather thick.

Color characters: Considerably lighter than specimens of the first instar, although still of a somewhat greenish shade.

THIRD INSTAR.—Morphological characters. Antennal segments as follows: III, 0.2 to 0.224 mm., average 0.213 mm.; IV, 0.096 to 0.112 mm., average 0.106 mm.; V, (0.072 plus 0.16 mm.) to (0.088 plus 0.208 mm.), average (0.08 plus 0,177 mm.). In this instar Segment III sometimes shows division, in which case Segments III and IV have about the following proportions: III, 0.144 mm.; IV, 0.112 mm. Eyes with a large number of facets; cornicles still stout and measuring 0.144 to 0.176 mm., average 0.16 mm.

Color characters: Approaching those of the adult insect.

FOURTH INSTAR.—Morphological characters. Antennal segments as follows: III, 0.192 to 0.24 mm., average 0.216 mm.; IV, 0.112 to 0.16 mm., average 0.128 mm.; V, 0.128 to 0.152 mm., average 0.136 mm.; VI, (0.08 plus 0.184 mm.) to (0.112 plus 0.24 mm.), average (0.096 plus 0.213 mm.). Cornicles 0.1224 to 0.256 mm., average 0.227 mm.

Color characters: Almost those of the adult insect.

FIFTH INSTAR.—Morphological characters. Antennal segments as follows: III, 0.336 to 0.384 mm., average 0.352 mm.; IV, 0.192 to 0.224 mm., average 0.2 mm.; V, 0.144 to 0.176 mm., average 0.16 mm.; VI, base, 0.096 to 0.112 mm., average 0.099 mm.; unguis 0 192 to 0.224 mm., average 0.208 mm. Segments imbricated, the first segment being distinctly ridged on its inner margin and armed with a number of slightly capitate hairs. Antennæ on frontal tubercles, these being armed on their inner edge with slightly capitate hairs; vertex slightly protruding; crown armed with numerous hairs and near its caudal margin with a pair of distinct tubercles (in many specimens only one of this pair present and in other specimens neither); eyes prominent, their tubercles small; prothoracic tubercles not as prominent as in the later forms; abdomen with distinct lateral tubercles and fine hairs; the last two abdominal segments each with a pair of distinct dorsal tubercles; cornicles subcylindric, 0.288 to 0.32 mm., average 0.305 mm., considerably broader at the base than at the apex, slightly flanged and strongly imbricated; cauda conical, short, densely setose and covered with numerous long hairs; anal plate rounded and similarly armed; legs with short stiff hairs, femora rough and covered with sensory-like markings, hind tibiæ 0.83 mm. long; form very globose, the abdomen not showing segmentation; length from vertex to tip of cauda 1.68 mm; width of abdomen 1.44 mm.

Color characters: General color reddish or purplish brown, dusted with a bluish white powder. Antennæ, with the exception of the basal portion of Segment III and sometimes Segment II, black; head and prothorax dark brown or blackish; abdomen varying shades of brown or purplish with a few minute dark markings which include the lateral tubercles; cauda and anal and genital plates black; cornicles black; legs entirely black with the exception of a light ring at the base of each femur; color between and surrounding the cornicles rusty.

Location: Found within the curled leaves of the apple, usually entirely hidden by the tightly rolled leaf.

LENGTH OF NYMPHAL LIFE

When newly hatched, the stem mother wanders about on the twig until a bud is reached. Here she settles and commences feeding, crowding down into the bursting bud. Before feeding she has a wrinkled appearance, but begins to fill out in a day or two.

The duration of the first instar is considerably longer than that of the following ones, but this depends a good deal on weather conditions. If

the weather is favorable, the first instar will be much shortened. The total nymphal life averaged in the experiments 15 days, and this was the average in spite of the variation in the length of the instars.

REPRODUCTION

The stem mothers began reproducing in the experiments during the 24 hours following the last molt. Considerable variation was noted amongst individuals, both as to the total number of young produced and the number produced daily. Reproduction in groups, as has been noted by the authors for *Aphis pomi*, is very common with this species also. The greatest number of young produced by any individual stem mother was 260, and these were produced during a period of 20 days. The smallest number produced by one adult was 81 in 24 days. The average number for 12 individuals was 71.1 young each, the average reproductive period being 26.3 days. The average daily production was 6.3 per insect and the greatest record was 14.6, one female giving birth to 44 young in 3 days.

LONGEVITY

The greatest length of life observed was 45 days. Many other stem mothers which died from accident before an equally long period of time were in very good condition at the time of their death. It would seem, therefore, that this period is not very far above the average.

SPRING FORMS

Several generations of aphids, in which both wingless and winged forms occur, follow the stem mother upon apple. In 1914 five apple-infesting generations occurred in the experiments, while in 1915 seven such generations were observed. In both cases the first stem-mother generation is not included in the figures given.

The first of these generations, the second generation from the egg, appears to be composed entirely of wingless insects—at least there were no winged forms in the experiments, either in 1914 or 1915. In the third generation, however, a few winged forms occurred and the percentage of winged to wingless insects increased rapidly up to the sixth generation in 1914 and the eighth in 1915, in which all the adults had wings. Two intermediates were also reared with the spring migrants.

SPRING WINGLESS VIVIPAROUS FEMALE

DESCRIPTION

FIRST INSTAR.—Morphological characters. Antennæ with the following measurements: Segment III, 0.16 to 0.208 mm., average 0.182 mm.; IV, (0.048 plus 0.176 mm.) to (0.072 plus 0.224 mm.), average (0.059 plus 0.204 mm.). Cornicles about 0.088 mm., rather short and thick.

Color characters: General color light yellow; about the base of each cornicle a small reddish patch. Eyes dark red or brownish; cornicles dusky. Legs pale yellow with the tarsi and possibly the distal extremities of the tibiæ dusky to black. Antennæ

with the proximal half pale yellow and the distal half dusky or black. Labium pale yellow tipped with dusky brown; body somewhat pulverulent.

SECOND INSTAR.—Morphological characters. Antennæ with the following measurements: Segment III, 0.192 to 0.232 mm., average 0.212 mm.; IV, 0.096 to 0.112 mm., average 0.104 mm.; V, (0.064 plus 0.256 mm.) to (0.08 plus 0.288 mm.), average (0.067 plus 0.272 mm.). Cornicles about 0.112 mm.

Color characters: Somewhat similar in color to the insects of the first instar, but somewhat more pinkish as compared to the distinct yellow of the first instar.

THIRD INSTAR.—Morphological characters. Antennæ with the following measurements: Segment III, 0.208 to 0.24 mm., average 0.22 mm.; IV, 0.160 to 0.192 mm., average 0.177 mm.; V, 0.128 to 0.150 mm., average 0.14 mm.; VI, (0.064 plus 0.288 mm.) to (0.088 plus 0.384 mm.), average (0.083 plus 0.342 mm.). Cornicles 0.176 to 0.224 mm., average 0.198 mm.

Color characters: Much darker than the earlier instars, taking on the rosy tint of the adult insects and the gray slaty powdering met with on the adult.

FOURTH INSTAR.—Morphological characters. Antennæ as follows: Segment III, 0.304 to 0.4 mm., average 0.352 mm.; IV, 0.208 to 0.328 mm., average 0.271 mm.; V, 0.144 to 0.208 mm., average 0.187 mm.; VI, (0.08 plus 0.352 mm.) to (0.102 plus 0.504 mm.), average (0.094 plus 0.433 mm.). Cornicles 0.24 to 0.288 mm., average 0.259 mm.

Color characters: Similar to the previous instar, although darker and more nearly like the adult.

FIFTH INSTAR (ADULT) (Pl. 21, C).—Morphological characters. Antennæ with the following measurements: Segment III, 0.43 to 0.656 mm., average 0.52 mm.; IV, 0.256 to 0.48 mm., average 0.348 mm.; V, 0.192 to 0.288 mm., average 0.235 mm.; VI, (0.096 plus 0.4 mm.) to (0.128 plus 0.592 mm.), average (0.108 plus 0.486 mm.). Cornicles 0.336 to 0.496 mm. Body rotund; upper surface of the head often with two rather prominent tubercles and a similar pair on the dorsum of the last two abdominal segments. Cauda short, abruptly conical, setose, cornicles distinctly flanged and slightly curved, imbricated. Antennæ not distinctly imbricated, except the distal segments. Labium extending to between the second and third pairs of coxæ. Lateral tubercles distinct.

Color characters: General color rosy brown, having a pinkish cast, owing to a powdery covering. Some of the older specimens are almost of a purplish color whereas younger specimens are decidedly reddish pink. Antennæ yellowish, with the distal extremity black. Legs yellowish, the tarsi and the distal extremities of the tibiæ black or brown. Labium tipped with brown. Cornicles and eyes black.

Location: Occurring in colonies within the curled leaves of the apple.

DURATION OF NYMPHAL STAGES

The average length of nymphal life of the wingless spring form is 9 to 10 days. This period is divided about equally among the four immature stages. Occasionally one stage will be a day or even more longer than the others, and such retardation of growth may occur in any one of the four stages. These variations are apparently due almost entirely to temperature conditions, although in the experiments occasional effects were due to poor food conditions.

REPRODUCTION

Reproduction commenced almost invariably about 24 hours after the insect had attained maturity and continued for a period of from 12 to 26

days. In the experiments, with one exception to be noted later, the adults died within one or two days after depositing their last young. The average number of young deposited by 14 females was 121.8 per insect. The greatest number deposited by one female was 180 young, the smallest 66 young. It should be noted that the female which deposited only 66 young was abnormal. The average daily production for the 14 insects was 5.7 per female. The greatest daily average for one female was 14.3 young, the mother producing 43 young in 3 days. One other aphid produced 60 young in 5 days. In a general way the aphids of the earlier generations produced more young than did those which came later. No exact statement can be made, however, as there was much variation in the matter.

LONGEVITY

The average total length of life of 13 of the aphids used in obtaining the data on reproduction was 31.4 days.

As has been previously stated, all but one of the insects in question died within one or two days after depositing their last young. One insect, however, gave birth to only 66 young and then lived for 11 days more without reproducing. This aphid was killed on the eleventh day and sectioned. When killed, the insect was apparently perfectly normal, except for the fact that the abdomen was darker in color than that of the other aphids. This dark color was diffused irregularly over the abdomen and appeared to be produced by some change within the body of the insect, rather than to be simply a case of melanism in the hypodermal coloring. The insect was very plump; in fact, much plumper than were other aphids which had produced even less young.

On sectioning it was found that there was an almost complete disintegration of the reproductive system, only small isolated portions being present. Moreover, there were the remnants of two half-grown embryos lying free in the coeloma. The fat body had made an excessive growth, almost completely filling the abdomen, and being abundant in the thorax. The digestive canal was apparently perfectly normal, as were the other organs of the body.

It is very interesting to note in this connection that three examples of a similar nature were found in experiments on the life history of *Aphis avenae*.

INTERMEDIATE FORM

During the spring of 1914 two intermediates (Pl. 21, D) of this species were reared. These two insects are of particular interest since the other intermediates reared have all occurred among the summer forms, the winged insects of which migrate, if at all, to other plants of the same species as those upon which the wingless insects feed. These two insects, however, were reared in the spring upon apple. Had they become

winged they would have been unable to exist upon apple, but must have flown to plantain. Any young which they might possibly have deposited upon apple would have died soon after birth.

The two intermediates in question continued feeding upon apple after becoming adult, produced their young normally, and these, in turn, produced spring migrants, which left the apple. In other words, the two intermediates not only evince a change of form from winged toward wingless, but also a like change in habit.

In discussing the intermediates of *Aphis pomi* the writers (9) have advanced the suggestion that the winged form and bisexual reproduction represent the more primitive condition among aphids and that these insects are at present in an active state of variation toward a wingless form and parthenogenetic reproduction. If this supposition is correct, the present examples would indicate that the alternation of hosts is a more primitive condition, even possibly that the aphids were originally general feeders and that some of them are varying toward forms which will feed only upon one host.

DESCRIPTION

Morphological characters: Antennæ of about equal proportions in both specimens. Segment III, 0.576 mm.; IV, 0.352 mm.; V, 0.288 mm.; VI, base 0.112 mm.; unguis, 0.576 mm. One specimen has 6 sensoria near the distal extremity of III, 15 on IV, and 3 on V. The other specimen has both antennæ with the following sensoria: 32 and 38 on Segment III, 17 and 21 on IV, 3 and 4 on V. Cornicles in one specimen 0.4 mm. long and in the other 0.448 mm. Winged thoracic characters absent. Wings represented by small padlike structures 0.32 mm. long in one specimen and 0.16 mm. long in the other.

Color characters: General appearance and color resembling those of the wingless form.

SPRING MIGRANT

DESCRIPTION

Since the forms of the first three instars show little difference between those which become wingless and those which become winged no description is here given of the first three instars of the spring migrant.

FOURTH INSTAR (PUPA).—Morphological characters. Antennæ with the following measurements: Segment III, 0.384 to 0.432 mm., average 0.414 mm.; IV, 0.24 to 0.32 mm., average 0.28 mm.; V, 0.176 to 0.216 mm., average 0.2 mm.; VI, (0.08 plus 0.4 mm.) to (0.112 plus 0.512 mm.), average (0.96 plus 0.433 mm.). Cornicles 0.256 to 0.32 mm., average 0.272 mm.

Color characters: Thorax and wing pads pink, shaded with dusky at the tips of the pads. Top of head and first two antennal segments bluish dusky; Segments III and IV of antennæ whitish, distal segments black. Abdomen slaty blue, the embryos showing through as yellowish white patches. The covering of mealy wax gives a grayish cast to the abdomen. Between the cornicles and caudad of them a dull rusty area, not a bright rusty area as in the wingless individuals. Lateral and caudal tubercles showing as minute dark brown spots. Cornicles brownish black. Legs whitish with the exception of the tarsi and the distal tips of the tibiæ. Labium tipped with black or dark brown. Eyes dark brown.

FIFTH INSTAR (ADULT) (Pl. 20, C).—Morphological characters. Antennæ as follows: Segment I, 0.08 mm.; II, 0.064 mm.; III, 0.56 to 0.72 mm., average, 0.676 mm.; IV, 0.368 to 0.464 mm., average, 0.419 mm.; V, 0.240 to 0.32 mm., average, 0.272 mm.; VI, (0.112 plus 0.504 mm.) to (0.128 plus 0.68 mm.), average (0.116 plus 0.582 mm.). Segments I and II strongly imbricated on their inner margins and armed with a few short spines. Segment III imbricated and armed with 53 to 60 oval, double rimmed, protruding sensoria which give the segment a knotted appearance. These are distributed over the entire segment; and a number of short spinelike hairs are also present. Segment IV similar to segment III but with from 22 to 29 sensoria. Segment V also similar, but the smaller number of sensoria (4 to 10) causing the imbrications to appear more distinct. The distal sensorium on this segment is the usual fringed one and not similar to the others on the segment. Segment VI strongly imbricated throughout and with the usual sensory group at the base of the unguis. Antennæ placed on small frontal tubercles which are notched within and armed with a few capitate hairs; vertex almost straight in some specimens, while the median ocellus protrudes in others; crown with a pair of tubercles placed between the compound eyes. This character is, however, not a constant one, as the writers have many specimens in which these tubercles are lacking. Prothorax with a prominent lateral tubercle on each side. In some specimens there are two of these tubercles on one side, and in the writers' specimens this seems always to be the left side. Forewing 2.48 mm. long and 1.008 mm. wide at its broadest part. Venation usually normal, first branch of media slightly nearer its insertion than it is to the tip, radial sector considerably curved, stigma 0.56 mm. long on the costal margin; hind wing 1.6 mm. long, hamuli 0.56 mm. from the distal extremity. Abdomen with distinct lateral tubercles and with prominent dorsal tubercles on the two caudal segments; cauda short, conical, setose, and armed with usually three pairs of long curved spinelike hairs; anal plate bluntly conical or rounded, densely setose, and armed with many curved spinelike hairs. Cornicles cylindric, slightly curved, faintly imbricated and distinctly flanged; length 0.288 to 0.384 mm.; legs slender, hind tibiæ 1.07 to 1.1 mm.; hind tarsus about 0.096 mm. long.

Color characters: General color brownish green, marked with black as follows: Head above uniform black, the prothorax and the thoracic shield black, the margins, however, near the wing insertions without black markings. Abdomen with a large black quadrate patch on middle of dorsum surrounded by a narrow unmarked area; cephalad of this patch there are often a number of small transverse markings, and caudad of it a semicircular black marking which includes the insertions of the cornicles; this is sometimes fused with the quadrate patch; margins of the abdomen with three large rounded black spots cephalad of the insertion of the cornicles and with sometimes a few smaller markings, lateral margins of the thorax with a large black spot. Below marked with black as follows: Vertex, trophic tubercle, and margins and tip of labium; a small spot on the prothorax; sternal plate; a band between and encircling the hind coxæ; cauda and anal and genital plates. Antennæ black; coxæ black, trochanter and proximal extremity of femur black; remainder of femur, the distal extremity of the tibia, the tarsus, and claws black; remainder of tibia yellowish brown; cornicles black. Wings clear, veins thin, stigma dusky.

Location: Found in the curled leaves of the apple and on the leaves and stems of the plantain reproducing.

LENGTH OF NYMPHAL LIFE

The winged form requires about two days more than the wingless one for its immature stages, though this period may vary in exceptional cases from 1 to 3 days. Thus, the total length of the period is from 11 to 13 days, the usual length being 12. The two extra days are spent in the fourth or pupal stage, the pupæ being from 2 to 3 days old when the wingless form becomes adult.

REPRODUCTION

The migrants appear to commence reproduction in about 1 to 2 days after settling upon the rib grass. The period for those transferred in the experiments varied from 1 to 5 days. It is very probable, however, that those insects which required the longer period were transferred too soon after becoming winged, since in the field the migrants usually remain on the apple for one or two days at least after the last molt.

Ten migrants produced an average of 18 young, varying from 10 to 29. The average reproduction period for these individuals was 5.8 days, varying from 3 to 9 days. The daily average was 3.2 young per female. It is very interesting to note that without exception the mothers brought forth more young on the first day of reproduction than on any of the following days, the numbers of young ranging, with one or two exceptions, from 7 to 10 for that day. It will be noted later that the reproduction of fall migrants resembles that of the spring winged form.

LONGEVITY

The total average length of life for the 10 insects which have just been mentioned was 25½ days. As stated when discussing the length of life of immature forms, the period spent on apple averages 12 days, plus 1 or 2 days as adults. The average length of life on rib grass was 12½ days, varying from 3 to 29. All but two of the insects observed lived for at least 2 days after producing their last young, while one lived in this way for 15 and another for 22 days. These conditions also will be found to exist in an exaggerated form among the fall migrants.

MIGRATIONS OF THE SPECIES

For many years entomologists have recognized the importance in the life history of this species of its secondary host. The actual discovery of this host was first made by Theodore Pergande, although no record was published. It would appear that Pergande observed the aphids under greenhouse conditions, for his first date referring to the plantain is January 25, 1882. His manuscript note reads as follows: "Large numbers of this aphid are noticed to-day on leaves of the narrow-leaved plantain. * * * They are found in all stages; larva, wingless females, pupæ and winged insects." Several of the specimens referred to were mounted on slides and these have been examined by the senior writer and are without a doubt *A. malifoliae.*

Since Pergande published no account of his findings, it had been believed by entomologists that the alternate host of the species had never been located. This is the view set forth in all the publications on the species. Ross (8, p. 23), however, reported his transfers made to *Plantago major* and *P. lanceolata;* and since his note appeared, these plants have become fairly well known as the alternate hosts of the species. The writers' observations on the species in connection with plantains were begun in 1913 when the senior writer made transfers to

broad-leaved plantain. Other transfers were made during 1914, but it was not until the spring of 1915 that a large and definite migration to plantains was noted in the field. Winged specimens were then observed alighting in numbers and reproducing upon the underside of the leaves and on the long flower stems of the rib grass (Pl. 23). Ross (8) reported that he was able to rear the species throughout the summer on apple. This the writers were unable to do even with a very few insects on a plant; all of the lines carried ultimately produced winged forms and migrated. It must be remembered also in this connection that the writers selected wingless and winged forms from each mother in order to obtain offspring. It is quite possible, however, that the lines carried by Ross had less of a tendency toward winged forms throughout than had those of the writers. Since the migration does not occur in any definite generation but is scattered throughout the apple life of the insect from the second generation onward, such a condition of affairs might easily occur. The following fact also is of importance here: The intermediates discovered by the writers remained upon apple and there reproduced, thus taking on the nature of the primary wingless forms. This would indicate that ultimately the species may become a permanent apple species like *Aphis pomi*, and the fact that Ross had carried it through the season upon apple would seem to show that this tendency is further advanced in the Ontario region where Ross conducted his experiments than in the Virginia region where the writers reared their insects.

The fact that spring migrants occurred in every generation on apple in the writers' experiments from the third to the eighth caused this migration to spread over a long period of time, from May 20 to July 1 at Vienna, Va. The pupæ of the spring migrants very often do not feed but may be found under loose bark and in the crevices of the limbs and trunk. Many, however, remain within the curled leaves. In these places the winged form is produced and in a few days it migrates to the rib grass and settles upon the underside of the leaves and on the flower stems. Here it produces its young. Occasionally some winged forms reproduce upon the broad-leaved plantain, but at Vienna this is rare as compared to the large number that migrate to the rib grass.

The fall migrants leave the plantains about 1 or 2 days after becoming adult, and fly to apple trees, where they settle upon the under surfaces of the leaves and commence feeding. Reproduction usually begins within 24 hours after the insect reaches the apple, though the migrants may feed for two or three days before producing young.

When mature, the males leave the plantain, flying to apple. If the oviparous females are not fully mature when the males arrive, the latter settle down beside them and feed until such time as copulation can take place.

Recently, since this paper was prepared, Brittain (12, p. 16, fig. 2) has reported his generation experiments on plantain.

SUMMER FORMS

The usual summer form of this species is wingless. In fact, among over 1,000 individuals reared to maturity during the summer only 6 specimens of the winged form were observed. One of these died when a pupa. This latter insect belonged to the sixth generation from the spring migrant. The other 5 insects were apparently members of the third generation on plantain, although this was ascertained with certainty for only one individual, the other form occurring in experiments which had been set aside merely to maintain a surplus stock of material. Only 2 of these winged insects reproduced, their progeny being normal summer wingless aphids. Thus, while the winged insects may occur on plantain they are of no particular importance in the life history of the species.

It should be noted in connection with this form that many wingless insects were reared on the plants in the generations with these winged aphids and in the generations preceding and following them, the ratio being over 100 to 1. The production of this form can hardly have been due, therefore, to food conditions.

A maximum of 14 generations of the summer form were reared on plantain, with a possible theoretical minimum of 4 generations.

SUMMER WINGLESS VIVIPAROUS FEMALE

DESCRIPTION

FIRST INSTAR.—Morphological characters. Antennæ with the following measurements: Segment III, 0.176 to 0.224 mm., average 0.196 mm.; IV, (0.048 plus 0.208 mm.) to (0.064 plus 0.248 mm.), average (0.056 plus 0.238 mm.). Cornicles 0.08 to 0.096 mm., average 0.084 mm.

Color characters: Pale greenish yellow with ultimately a rusty color across the abdomen between the cornicles. The color sometimes extends into the cornicles.

SECOND INSTAR.—Morphological characters. Antennæ with the following measurements: Segment III, 0.16 to 0.24 mm., average 0.209 mm.; IV, 0.102 to 0.128 mm., average 0.107 mm.; V, (0.064 plus 0.24 mm.) to (0.072 plus 0.32 mm.), average (0.065 plus 0.288 mm.). Cornicles 0.112 to 0.144 mm., average 0.136 mm.

Color characters: Similar to the specimens of the first instar.

THIRD INSTAR.—Morphological characters. Antennæ with the following measurements: Segment III, 0.128 to 0.176 mm., average 0.164 mm.; IV, 0.128 to 0.184 mm., average 0.156 mm.; V, 0.128 to 0.144 mm., average 0.136 mm.; VI, (0.064 plus 0.288 mm.) to (0.088 plus 0.392 mm.), average (0.076 plus 0.342 mm.). Cornicles 0.0176 to 0.208 mm., average 0.19 mm.

Color characters: Similar to those of the second instar.

FOURTH INSTAR.—Morphological characters. Antennæ with the following measurements: Segment III, 0.24 to 0.288 mm., average 0.264 mm.; IV, 0.176 to 0.24 mm., average 0.201 mm.; V, 0.144 to 0.192 mm., average 0.166 mm.; VI, (0.08 plus 0.36 mm.) to (0.096 plus 0.424 mm.), average (0.086 plus 0.39 mm.). Cornicles 0.24 to 0.272 mm., average 0.265 mm.

Color characters: The same as those of the adult form.

FIFTH INSTAR (ADULT) (Pl. 22, A).—Morphological characters. Antennæ slender, extending beyond the tips of the cornicles; measurements as follows: Segment III, 0.416 to 0.512 mm., average 0.454 mm.; IV, 0.256 to 0.368 mm., average 0.305 mm.;

V, 0.192 to 0.256 mm., average 0.225 mm.; VI, (0.088 plus 0.368 mm.) to (0.112 plus 0.512 mm.), average (0.097 to 0.441 mm.); segments imbricated, armed with a few hairs, but without sensoria. Antennal tubercles distinct; head without dorsal tubercles met with in the wingless form from apple. Legs slender; hind tibia 0.656 to 0.784 mm. long. Cornicles very slender, slightly curved, distinctly flanged and imbricated; length, 0.384 to 0.464 mm., average 0.443 mm. Abdomen without the prominent caudal tubercles met with in the apple form; cauda conical, rather elongate, more slender than that of the apple form, armed with three pairs of hairs and imbricated by rows of minute setæ. Form more elongate than the spring wingless forms, 1.36 by 0.8 mm.

Color characters: Color creamy-yellow with a slight brownish or even purplish cast; eyes dark brown; distal portions of antennæ, tips of cornicles, tip of labium, and tarsi brown or dusky. Between the cornicles and inclosing their insertions is a band of rusty red, this color sometimes also extending into the proximal portion of the cornicles; the red eyes of the embryos showing through the abdomen of the adult.

Location: Found in colonies on the under surface of the leaves of rib grass (*Plantago lanceolata*) and also on the flower stems; rarely also on other species of the genus.

DURATION OF NYMPHAL STAGES

This form of the species became adult in from 8 to 12 days, the period varying with the prevailing temperature. Insects born from the 10th to the 20th of June required 10 days for this period, while those born in July matured in 8 to 9 days. Later the period increased again to 12 days in September.

REPRODUCTION

The average number of young produced by 35 adults was 65.2, the maximum being 108 (produced by two insects) and the minimum 12. The average length of the reproductive period was 19 days, thus varying from 5 to 35 days. The average daily production of these 35 adults was 3.4 young per mother. The greatest average daily production for one adult was 5.4, this insect producing 43 young in 8 days; the lowest was 2.2, 77 young in 35 days. One insect brought forth 14 young in one day while 8 produced 10 or more in a like period. Of the two winged insects which reproduced, one brought forth 21 young in 7 days, and the other 13 young in 3 days. The first of the two lived for a few days after reproduction ceased.

LONGEVITY

The average total length of life for this form was 28 days, varying from 14 to 45 days. In general the insects born during the early part of the summer, particularly in June, were longer lived than any which followed, the latter all equaling or exceeding the average for the entire summer.

SUMMER WINGED VIVIPAROUS FEMALE

The early instars of this form are very similar to those of the summer wingless viviparous female and those of the fall migrant. A description of the fifth instar (adult) only will be given here. Since the form was

of very rare occurrence throughout the summer, it is not worth while to give averages for these very few insects.

FIFTH INSTAR (ADULT).—Morphological characters. Antennæ with the following measurements: Segment III, 0.688 mm.; IV, 0.496 mm.; V, 0.304 mm.; VI (0.128 plus 0.648 mm.). Cornicles 0.352 mm. Segment III with about 58 circular sensoria, IV with 34 similar ones, V with 6 or 8 arranged evenly along the segment. Head with tubercles above and the last two segments of the abdomen with a pair of dorsal tubercles.

Color characters: Similar to those of the spring migrant, the abdomen above nearly uniform brownish black or with a distinct patch.

DIMORPHIC REPRODUCTION

The writers have already noted in two other species of aphids, *A. pomi* and *A. avenae*, that the late summer and early fall individuals of the summer form may produce two forms of young. In *A. malifoliae* both summer wingless and fall migrants, or fall migrants and males, may be produced by one mother. In the experiments the former combination occurred, in a general way, earlier than did the latter. Records of the production of all three forms by one mother were not obtained.

As we found to be the case with *A. avenae*, when one mother produced both fall migrants and males, the latter were usually her last progeny.

FALL FORMS

Among the fall forms may be included the fall migrant which leaves the plantains and flies to apple, the oviparous females which are deposited upon the apple leaves by these fall migrants, and the males which fly from the plantains to fertilize the oviparous females.

FALL MIGRANT

DESCRIPTION

The early instars of the fall migrant are practically the same as those of the summer wingless form. It is therefore unnecessary to give descriptions of any instars excepting those following:

FOURTH INSTAR (PUPA).—Morphological characters. Antennæ with the following measurements: Segment III, 0.4 to 0.464 mm., average 0.427 mm.; IV, 0.288 to 0.344 mm., average 0.315 mm.; V, 0.208 to 0.24 mm., average 0.227 mm.; VI, (0.096 plus 0.488 mm.) to (0.112 plus 0.56 mm.), average (0.107 plus 0.518 mm.). Cornicles 0.288 to 0.320 mm., average 0.305 mm.

Color characters: Similar to the pupæ of the spring migrant.

FIFTH INSTAR (ADULT) (Pl. 21, A).—Morphological characters. Antennæ with the following measurements: Segment III, 0.624 to 0.800 mm., average 0.689 mm.; IV, 0.376 to 0.528 mm., average 0.472 mm.; V, 0.256 to 0.336 mm., average 0.308 mm.; VI, (0.112 plus 0.56 mm.) to (0.152 plus 0.712 mm.), average (0.132 plus 0.632 mm.). Segment III with about 60 circular elevated sensoria, IV with about 27, V with about 6 sensoria. Head without tubercles above as is usual in the spring migrant. Prothorax with very small lateral tubercles. Abdomen also with very small ones. Cornicles 0.304 to 0.384 mm., average 0.348 mm. Cauda conical, short; last two segments of the abdomen without tubercles above as in the spring migrant. Otherwise as in that form.

Color characters: Similar to those of the spring migrant. Abdomen usually with one large central black patch, three or four marginal patches, and a transverse band caudad of the cornicles, this band extending cephalad at its edges to touch the base of the cornicles. In some specimens the whole of the abdomen appears a uniform black and the legs also do not show the yellow regions usually met with.

Location: Upon the leaves of the plantain, usually upon the underside, and upon the underside of the leaves of the apple, depositing young oviparous females.

LENGTH OF NYMPHAL PERIOD

The length of the nymphal period varied from 13 or 14 days in early September to as much as 24 days for aphids born in October. The average period was 16 to 18 days. No figures for the various instars can be given since they varied greatly with the temperature, so that, while in one case the first instar might be the longest, in others the third or fourth would be.

REPRODUCTION

Sixteen fall migrants produced 114 young, an average of 7.1 young per mother. The greatest number produced by one mother was 13 (or more), two insects together producing 25; the smallest number was 3. The average length of the reproductive period of 25 insects was 5 days, varying from 1 to 10 days. In every case all but one or two of the young were produced within a period of 1 to at most 2 days. Later at periods varying from 3 to 8 days one or two more young might be produced.

LONGEVITY

The adults of this form usually lived for a considerable period after reproduction ceased, this period varying from 2 to (in one case) over 40 days.[1] The average length of this post-reproductive period for 15 insects was 23.6 days.

The longest total life recorded was 62 days, while the average was about 45 days.

The conditions with regard to the rate of oviposition found to exist among both spring and fall migrants are very interesting, since they are the exact opposite of the theoretical conditions for insects. The general statement is commonly made that those females which produce all their eggs (or progeny) in a short period die very soon afterwards, while the females which live for a long time are those which produce only a few eggs (or young) daily. In this case, however, the adults produce their young in a short period and then proceed to live for many days. The theory is based, in part at least, on the proposition that the insects which produce all their offspring or progeny within a short period become exhausted, and, of course, with these migrants only a small number of young are produced. Still, in so far as the general theory endeavors to explain the causes of a long adult life, it fails for these forms.

[1] Experiment closed before insect died.

MALE

DESCRIPTION

As with the fall migrant, the earlier instars of the male are similar to those of the wingless viviparous form; only the fourth and fifth instars, therefore, are here described.

FOURTH INSTAR (PUPA).—Morphological characters. Antennæ with the following measurements: Segment III, 0.416 mm.; IV, 0.32 mm.; V, 0.216 mm.; VI, (0.092 plus 0.504 mm.). Segment III with about 58 sensoria, IV with about 27, V with about 7. Cornicles about 0.284 mm.

Color characters: Somewhat similar to those of the spring pupa.

FIFTH INSTAR (ADULT) (Pl. 21, B).—Morphological characters. Antennæ with the following average measurements: Segment III, 0.728 mm.; IV, 0.462 mm.; V, 0.302 mm.; VI, (0.113 plus 0.657 mm.). Cornicles 0.304 mm., slightly thicker than those of the migrants. Head without tubercles above, prothorax with very minute lateral tubercles, sometimes with none visible; lateral abdominal tubercles very small; last two segments of abdomen without tubercles above as in the spring migrant. In this respect the male is like the fall migrant.

Color characters: Similar to those of the fall migrant. In many cases the large central abdominal spot is broken into a number of irregular bands and the crossband caudad of the cornicles is often divided, the total forming a series of cross stripes on the abdomen.

PRODUCTION

As has been noted previously, the males, when produced by a mother also bearing fall migrants, were usually the last progeny of the mother. The few mothers which produced only males were sisters of fall migrants. In this way the production of males commenced at about the same time as did that of the oviparous females.

NYMPHAL STAGES

The period spent in the immature stages varied from about 20 to 25 days. No general statement can be made as to the duration of the various instars, since these varied greatly among the individual insects.

MIGRATION

When mature, the males leave the plantain, flying to apple. If the oviparous females are not fully mature the males usually settle down beside them and feed.

OVIPAROUS FEMALE

DESCRIPTION

FIRST INSTAR.—Morphological characters. Antennæ with the following measurements: Segment III, 0.144 to 0.16 mm., average 0.148 mm.; IV, (0.048 plus 0.184 mm.) to (0.056 plus 0.216 mm.), average (0.049 plus 0.196 mm.). Cornicles 0.056 mm.

Color characters: Very pale greenish yellow with brown eyes.

SECOND INSTAR.—Morphological characters. Antennæ with the following measurements: Segment III, 0.136 to 0.16 mm., average 0.142 mm.; IV, 0.064 to 0.08 mm., average 0.07 mm.; V, (0.056 plus 0.216 mm.) to (0.064 plus 0.248 mm.), average (0.059 plus 0.232 mm.). Cornicles 0.08 mm.

Color characters: Slightly darker than in the first instar.

THIRD INSTAR.—Morphological characters. Antennæ with the following measurements. Segment III, 0.192 to 0.224 mm., average 0.206 mm.; IV, 0.088 to 0.104 mm., average 0.096 mm.; V, (0.064 plus 0.264 mm.) to (0.072 plus 0.296 mm.), average (0.065 plus 0.273 mm.). Cornicles 0.112 mm.

Color characters: Somewhat more orange-yellow than the earlier instars, with more or less distinct brownish areas about the bases of the cornicles.

FOURTH INSTAR.—Morphological characters. Antennæ with the following measurements. Segment III, 0.144 to 0.184 mm., average 0.169 mm.; IV, 0.112 to 0.144 mm., average 0.128 mm.; V, 0.112 to 0.128 mm., average 0.12 mm.; VI, (0.064 plus 0.296 mm.) to (0.08 plus 0.344 mm.), average (0.075 plus 0.31 mm.). Cornicles 0.144 mm.

Color characters: General color yellow-orange, with brownish red patches around the bases of the cornicles; head grayish, eyes brown to black.

FIFTH INSTAR, ADULT (Pl. 22, B).—Morphological characters. Antennæ with the following measurements: Segment III, 0.208 to 0.256 mm., average 0.241 mm.; IV, 0.152 to 0.192 mm., average 0.169 mm.; V, 0.128 to 0.16 mm., average 0.142 mm.; VI, (0.08 plus 0.304 mm.) to (0.088 plus 0.36 mm.), average (0.081 plus 0.329 mm.). Cornicles 0.192 to 0.216, average 0.204 mm. Antennæ without sensoria. Head occasionally with a pair of tubercles above but the last two segments of the abdomen without such tubercles. Cauda short and abruptly conical.

Color characters: Similar to those given for the previous instar excepting that the colors are a little more distinct. The eggs show through the body as dark areas.

Location: Found on the underside of the apple leaves feeding, or on the twigs or in the axils of the buds depositing eggs.

NYMPHAL STAGES

The oviparous females required a period of from 20 to about 28 days for the immature stages. As with the males, the duration of the various instars varied greatly with the individuals, the principal cause of this being the variation of the prevailing temperatures.

MATING

Mating occurred mostly on the twigs. Males mated with several females and in some cases the oviparous females mated at least twice. As was the case with *A. avenae*, the male endeavors to copulate with every adult oviparous female he meets; and unless the female has just mated, she does not endeavor to hinder the male.

OVIPOSITION

Nineteen ovipara laid a total of 120 eggs, an average of 6.3 eggs per insect. Five of these oviparous females laid 36 eggs, an average of a little over 7 eggs each. The highest record for one oviparous female is, therefore, 8 eggs.

FEEDING HABITS

The results of the feeding of the rosy aphis are very noticeable, as the leaves are much curled thereby. (Pl. 24, B.) The young stem mothers crowd into the opening buds, and as the leaves grow they curl and twist about the insects. As young are produced these reach other leaves and

soon there is a large cluster of twisted leaves which is very conspicuous. In orchards composed of large trees these curled bunches are usually met with on the lower half of the trees and very often the small fruit spurs on the larger limbs in the body of the tree are affected. On very young trees, however, the feeding habits seem to differ in that the growing tips of the branches are attacked. This causes them, as they develop, to become twisted (Pl. 25, B), and as this growth hardens the limb is permanently deformed by being looped upon itself (Pl. 25, C). A branch will sometimes become looped several times, thus causing a much deformed tree unless the affected branches are cut out. In very young trees that are badly infested this is often a good portion of the tree.

The insects may also be found feeding upon the fruit, and this they cause to be reduced in size, irregular in shape, and somewhat gnarled, or more or less pitted (Pl. 25, A, D). In orchards composed of large trees this damage to the fruit is often a factor of considerable importance.

As has already been stated, the normal summer host of the species appears to be rib grass. During the season of 1915, however, the insects were reared successfully on broad-leaved plantain (*Plantago major*) for a period of two months. No record of the generations was kept in this case and no transfers were made. The plant finally died. At that time a strong healthy colony was living upon it and apparently under normal conditions would have survived until fall. In several other experiments, however, much difficulty was experienced in procuring successful transfers from the apple to this species of plantain. Rib grass appears to be the host preferred. This is borne out by an examination of the occurrence of the insects in orchard regions. Observations were made over a large extent of orchard territory. Wherever the rosy aphis was found to be very abundant the narrow-leaved plantain was common in and about the orchards, the worst infested orchards being full of the growing plantain.

The writers' observations upon the insects, both in the experiments and in the field, indicate that all parts of the rib grass are subject to attack. The insects appear to feed with equal readiness upon the leaves, the stem, and the flower stalks, and they may be found upon both sides of the leaves. The greater number of the insects, however, fed on the under surface of the leaves, especially along the veins.

In the fall the migrants alight on the underside of the apple leaves, and when the oviparous females are produced these feed also upon the underside of the leaves. The males, after migration from the plantains, often may be found feeding with immature oviparous forms. So far as the writers have been able to observe, these fall forms do not cause the leaves to curl and twist as do the spring forms.

SUMMARY OF LIFE HISTORY

The eggs of this species begin hatching early in April (about April 8 in 1915) and hatching ceases in about a week (April 16 in 1915). The first stem mothers begin reproduction about April 25. From 5 to 7 generations of the spring forms occur on apple in Virginia, although Ross reports the species all summer on apple in Ontario. The first generation is wingless. A few winged forms appear in the next generation and their percentage to the wingless insects increases steadily in each generation until finally all the insects produced become winged. Intermediates may also occur, these acquiring the wingless habits and behaving like wingless insects.

Migration to plantain commences about May 20, and most of the insects have left the apple by about June 20. A few may continue on apple till about July 1.

From 4 to 14 generations of the summer form occur at Vienna, Va. These insects are practically all wingless, only a few occasional winged insects appearing.

The first fall migrants become adult about the second week of September (Sept. 13 in 1915; these insects were born on Aug. 31). They remain on the trees until after November 1. (In the writers' experiments they were produced till a much later period, but in the field they succumb to prevailing low temperatures more quickly than do either oviparous females or males.)

Production of oviparous females commences about the middle to the 20th of September, but very few are produced till early in October and their production is at its height about the middle of that month. Males begin to appear early in October, at the time the oviparous females begin to become adults, and the males also are most numerous about the last of October and early in November.

Oviposition commences the middle of October and continues till the oviparous females are all dead. Some oviparous females may oviposit as late as the latter part of December in case excessive low temperatures have not occurred before that time.

The life history as summarized is for the vicinity of Washington, D. C.

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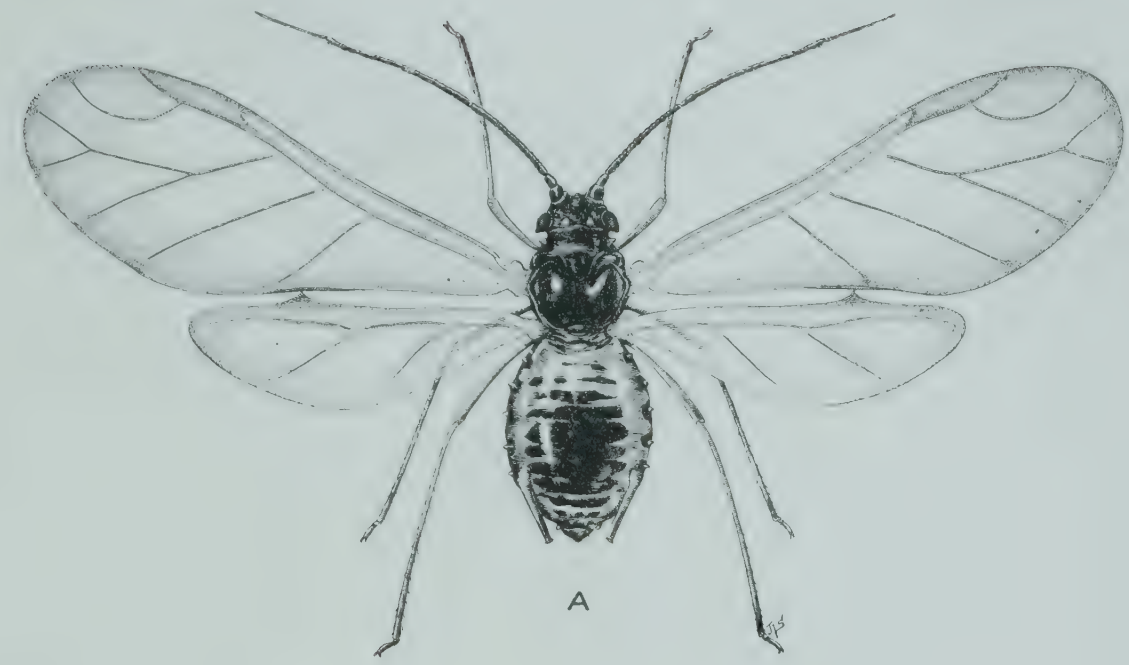
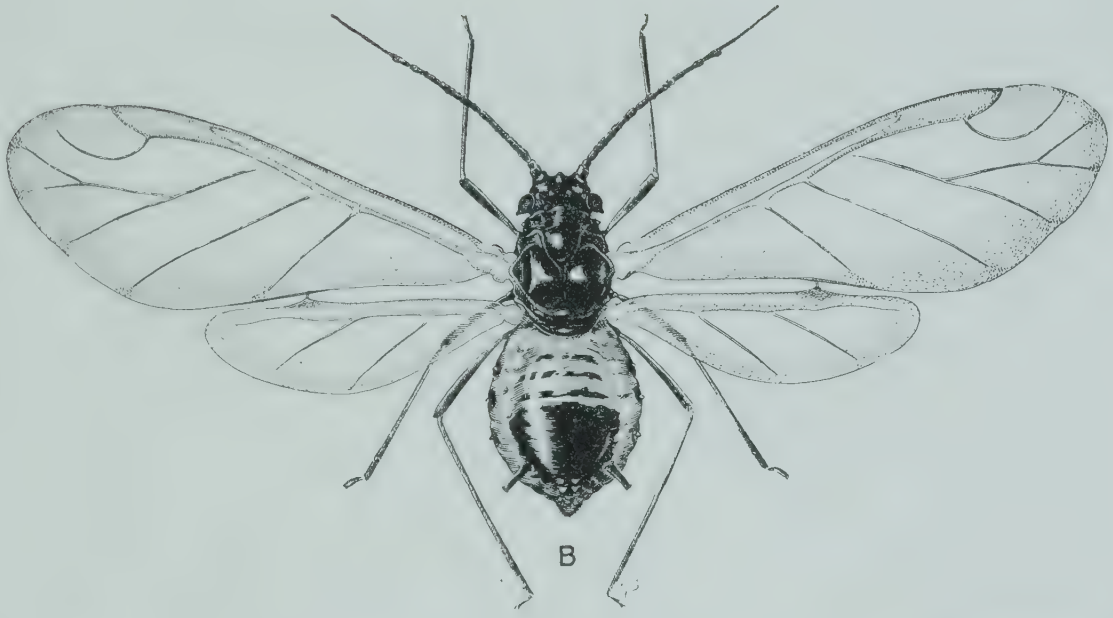
PLATE 20

A.—*Aphis sorbi:* Spring migrant.
B.—*Aphis kochii:* Spring migrant.
C.—*Aphis malifoliae:* Spring migrant.



A



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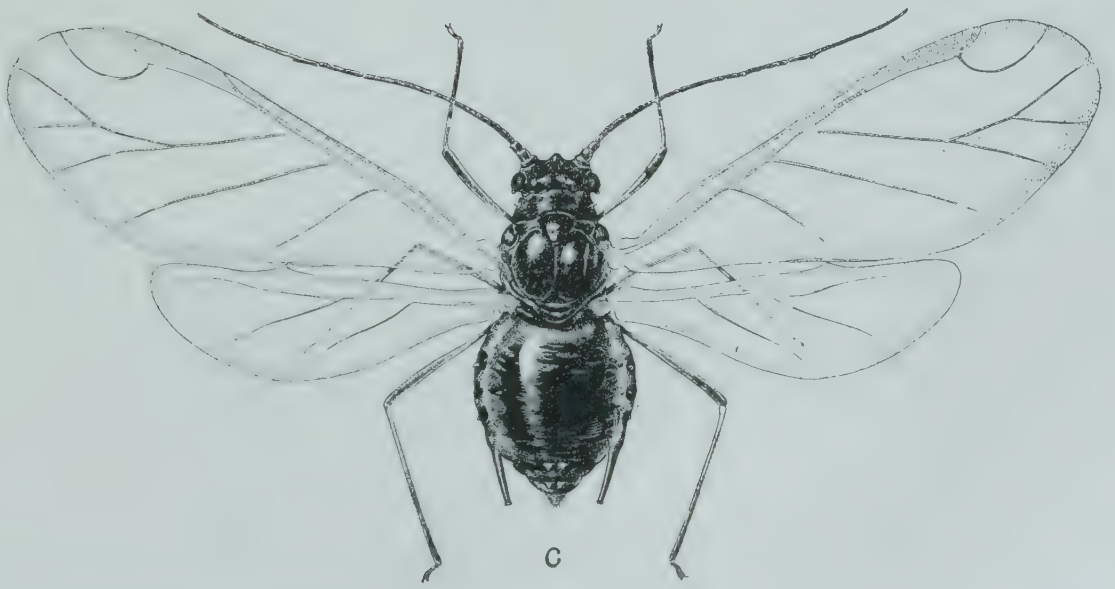


C

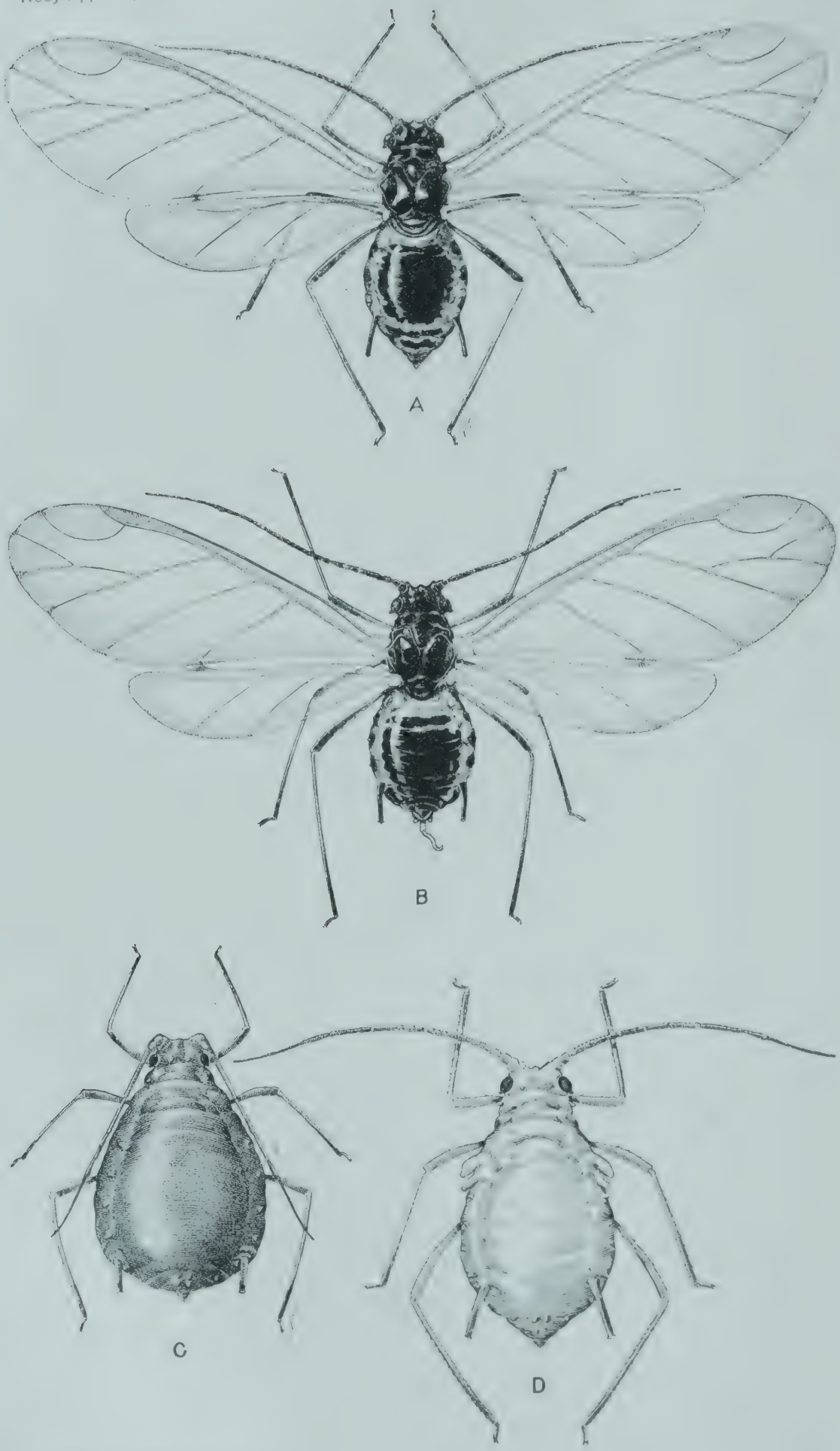

A
B
C
D

PLATE 21

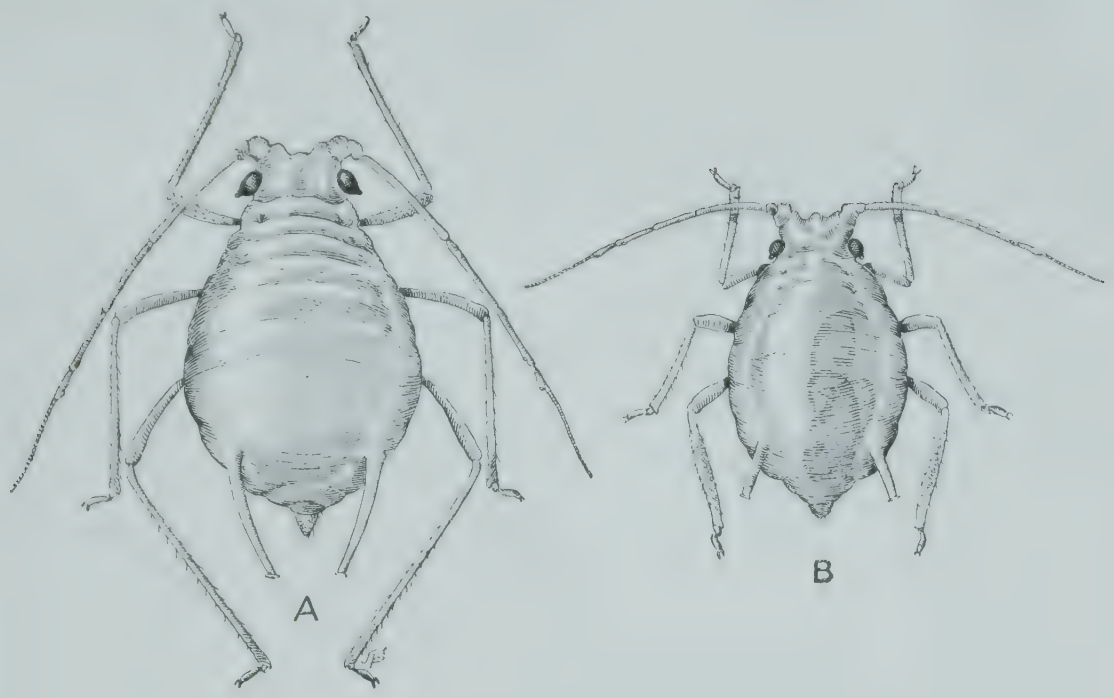
Aphis malifoliae:

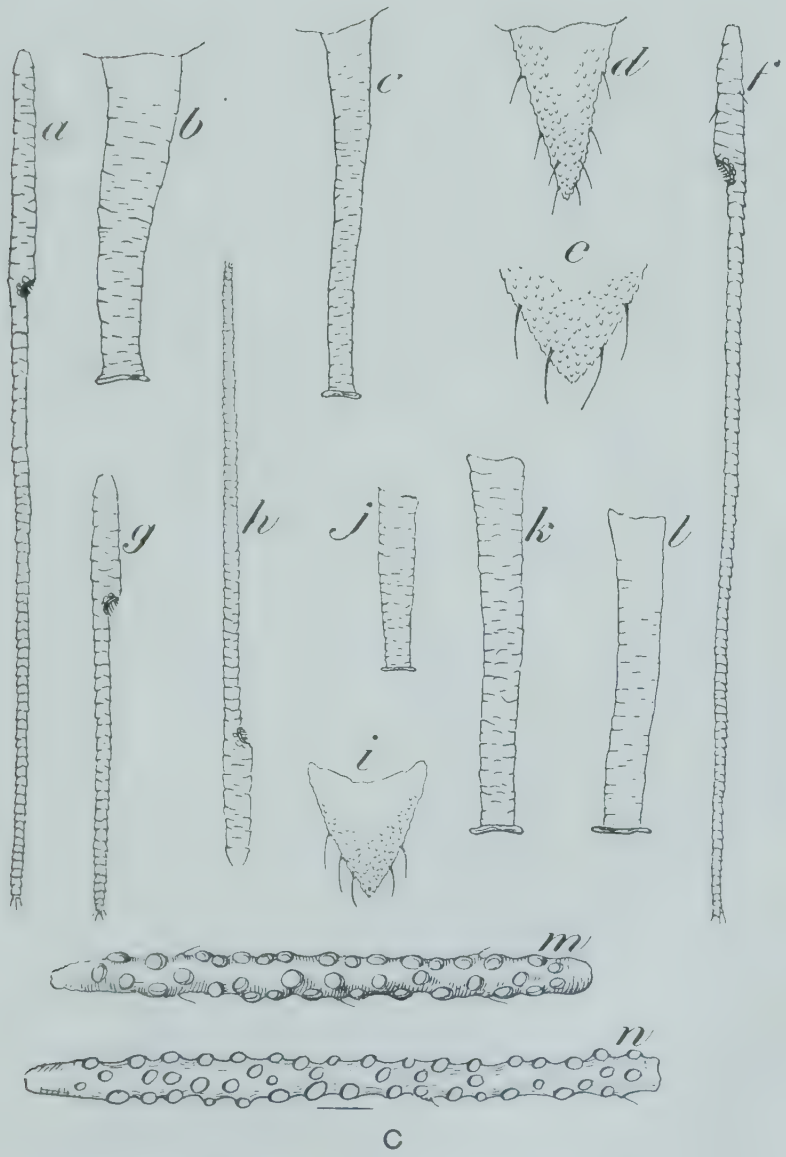
A.—Fall migrant.
B.—Male.
C.—Spring wingless female.
D.—Intermediate form.

PLATE 22

A.—*Aphis malifoliae:* Summer wingless form.
B.—*Aphis malifoliae:* Oviparous female.
C.—Structural details of *Aphis malifoliae, A. sorbi,* and *A. kochii.*

a, A. sorbi: Segment VI of antenna of winged form.
b, A. malifoliae: Cornicle of spring wingless form.
c, A. malifoliae: Cornicle of summer wingless form.
d, A. malifoliae: Cauda of summer wingless form.
e, A. malifoliae: Cauda of spring wingless form.
f, A. malifoliae: Segment VI of antenna of winged form.
g, A. malifoliae: Segment VI of antenna of stem mother.
h, A. kochii: Segment VI of antenna of winged form.
i, A. sorbi: Cauda of winged form.
j, A. kochii: Cornicle of spring migrant.
k, A. sorbi: Cornicle of spring migrant.
l, A. malifoliae: Cornicle of spring migrant.
m, A. sorbi: Segment III of antenna of spring migrant.
n, A. malifoliae: Segment III of antenna of spring migrant.


A
B


a
b
c
d
e
f
g
h
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j
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m
n
C

PLATE 23

Aphis malifoliae on its alternate host, *Plantago lanceolata*.

PLATE 24

A.—Broad-leaved plantain showing the effect of an attack by *Myzus plantaginis*.
B.—Apple leaves curled by colonies of *Aphis malifoliae*.

A

B

A
B
C
D

PLATE 25

A.—Rhode Island Greening apples deformed by *Aphis malifoliae.*
B.—Apple twigs twisted by colonies of *Aphis malifoliae:* Beginning of twisting.
C.—Apple twigs twisted by colonies of *Aphis malifoliae:* Twisted twig.
D.—Winesap apples deformed by *Aphis malifoliae.*

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USE OF TWO INDIRECT METHODS FOR THE DETERMINATION OF THE HYGROSCOPIC COEFFICIENTS OF SOILS[1]

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INTRODUCTION

The relative advantages and disadvantages of the moisture-equivalent method for the indirect determination of the hygroscopic coefficients of soils have been discussed in a previous article (3).[2] While in connection with soil-survey reports the moisture equivalents may conveniently be used to indicate the relative fineness of texture as a single-valued expression,[3] even the determination of these consumes far more time than is desirable in connection with many soil studies.

A very simple, rapid method seemed to be offered by a formula derived by Briggs and Shantz (4, p. 66) for the estimation of the hygroscopic coefficient from the maximum water capacity, as determined by Hilgard's method. Such an indirect method would prove extremely useful if it could be relied upon to give results in at least fair accord with those obtained by direct determination. The determination of the maximum water capacity by Hilgard's method requires only very simple apparatus, consumes but little time, and can be carried out in the most poorly equipped laboratory and doubtless even in an ordinary farm kitchen (14). To test the reliability of this proposed method, we have made determinations of the water capacity of 53 soils of which the hygroscopic coefficients had previously been carefully determined.

[1] The work reported in this paper was carried out in 1912 at the Nebraska Agricultural Experiment Station, where the authors were, respectively, Chemist and Assistant in Chemistry.

[2] Reference is made by number to "Literature cited," p. 359.

[3] The advantages of such a single-valued expression of the "different degrees of 'heaviness' of soils" (7, p. 440) appears to have first been suggested by Hilgard in connection with his introduction of the determination of the hygroscopic coefficient (6, p. XI).

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A method which will give results sufficiently reliable and accurate for many purposes, such as soil surveys, and yet be far more simple even than the preceding and more economical of time where any very large number of samples is to be handled, seems capable of development. This method would be an indirect one based upon the determination of the hygroscopic moisture in air-dried samples. As our work on this was interrupted several years ago by the removal from Nebraska first of the junior author and later of the senior author and there appears little probability of either of them being able to continue the work, at least for some time, the results are reported in the hope that in some other laboratory the limitations of the method may soon be determined.

HISTORICAL REVIEW

Hilgard's method for the determination of the maximum water capacity first proposed in 1893 (8, p. 256; 9, p. 74) has later been described in various publications (12, p. 82; 10, p. 15; 11, p. 209). From a comparison of the hygroscopic coefficient with the wilting coefficient in the case of a series of 17 soils and of the maximum water capacity with the wilting coefficient in the case of another series of some 15 soils Briggs and Shantz (4) have derived the formula:

$$\text{Hygroscopic coefficient} = (\text{maximum water capacity} - 21) \times 0.234$$

Unfortunately only four of the samples appear in both series. The data on these are reported in Table I. The calculated values agree fairly satisfactorily with those directly determined.

TABLE I.—*Relation of the maximum water capacity to the hygroscopic coefficient as found by Briggs and Shantz*

Soil No.	Type of soil.	Maximum water capacity.	Hygroscopic coefficient.		Difference.
			Determined.	Calculated.	
7	Coarse sand	23.2	0.5	0.5	0.0
2	Fine sand	29.9	1.5	2.1	+ .6
8	.....do	28.5	2.3	1.7	− .6
9	.....do	31.4	2.3	2.4	+ .1

Briggs and Shantz appear to have overlooked the work of Loughridge (12), who, some 20 years previously, using some 40 California soils ranging in texture from clays to sands, made a critical study of the relation of both the maximum water capacity and the hygroscopic coefficient to the mechanical composition. In the case of all of these he determined the hygroscopic coefficient, the maximum water capacity, both by weight and by volume, and the mechanical composition. In the first three columns of Table II we give the portions of his data dealing with the present subject, arranging the soils in the order of their hygro-

scopic coefficients. In the fourth column are shown the hygroscopic coefficients calculated from the maximum water capacities by the Briggs-Shantz formula, and in the last the differences between these and the directly determined values. While with about half the soils there is a fair agreement, in the case of the others the divergence is so wide that the calculated values would be quite misleading as to the relative hygroscopicity.

TABLE II.—*Concordance of the determined hygroscopic coefficient with that calculated by the Briggs-Shantz formula from the maximum water capacity in the case of California soils reported by Loughridge*

Soil No.	Maximum water capacity.	Hygroscopic coefficient.		Departure.
		Determined.	Calculated.[a]	
6	54.9	14.53	7.93	−6.60
863	68.8	14.20	11.18	−3.02
188	59.6	13.70	9.03	−4.67
643	64.6	13.51	10.20	−3.31
1679	46.0	11.98	5.85	−6.13
68	59.8	11.19	9.08	−2.11
676	54.4	11.11	7.82	−3.29
77	68.9	10.38	11.21	.83
110	52.4	10.32	7.35	−2.97
67	50.7	10.26	6.95	−3.31
789	57.8	9.74	8.61	−1.13
506	55.6	9.26	8.10	−1.16
8	52.1	9.18	7.28	−1.90
10	82.3	9.18	14.34	5.16
1	63.5	7.52	9.95	2.43
1678	34.2	6.41	3.09	−3.32
1113	52.3	6.00	7.32	1.32
1115	53.8	5.93	7.68	1.75
1284	58.8	5.81	8.85	3.04
168	38.7	5.49	4.14	−1.35
1167	41.7	5.38	4.84	−.54
1645	58.4	5.27	8.75	3.48
4	46.6	5.18	5.99	.81
2	52.4	5.14	7.35	2.21
1647	49.9	5.14	6.76	−1.62
986	42.0	5.10	4.91	−.19
1663	48.0	5.06	6.32	1.26
586	47.3	4.86	6.15	1.29
1655	44.3	4.20	5.45	1.25
1159	37.4	4.00	3.84	−.16
1055	36.9	3.62	3.72	.10
1197	34.8	3.48	3.23	−.25
1148	41.5	3.43	4.80	1.37
51	49.5	3.17	6.70	3.53
9	58.9	2.63	8.87	6.24
130	40.7	2.30	4.61	2.31
1281	37.9	1.98	3.95	1.97
1147	28.7	1.84	1.80	−.04
1595	29.7	1.15	2.04	.89
233	23.0	.79	.47	−.32

[a] By the Briggs-Shantz formula: Hygroscopic coefficient=(maximum water capacity−21)×0.234.

Caldwell (5, p. 15) in connection with a study of the permanent wilting in plants has determined both the hygroscopic coefficients and the water capacities of "a very pure sand, an 'adobe' clay loam containing less than 0.1 per cent of humus, and nineteen artificial mixtures of these two soils varying by increments of 5 per cent of loam from 95 per cent sand with 5 per cent loam to 95 per cent loam with 5 per cent sand." The observed hygroscopic coefficients of the two soils were 2.41 and 7.51 and the corresponding values calculated from the water capacities 2.30 and 7.40, respectively. The calculated coefficients of the artificial mixtures agreed equally well with the directly determined values. Clearly the concordance is here well within the limits of experimental error; and if the same held true for a series of 21 natural soils with a range in hygroscopic coefficient from 2.5 to 7.5, this indirect method would leave little to be desired in respect to simplicity and reliability.

It must, however, be emphasized that such artificial mixtures can not take the place of natural soils in testing such a method, as, if a formula holds true on each of two components separately, it should hold equally true for all mixtures of these. Thus, to illustrate, in the case of the soils studied by Loughridge (12), No. 77 and 1147, with determined hygroscopic coefficients of 10.38 and 1.84 and calculated values of 11.21 and 1.80, respectively, might be employed to prepare artificial mixtures and all the latter should show water capacities such that the calculated hygroscopic coefficients should agree with those directly determined, while with similar mixtures of No. 643 and 51, with determined values of 13.51 and 3.17 and calculated values of 10.20 and 6.70, respectively, the intermediate mixtures should be expected to show a concordance, although neither of the two soils by itself did.

ESTIMATION OF THE HYGROSCOPIC COEFFICIENT FROM THE MAXIMUM WATER CAPACITY

The determinations of the maximum water capacity were made in triplicate or quadruplicate, the concordance of the determinations being shown in Table III.

TABLE III.—*Concordance of quadruplicate determination of the maximum water capacity*

Soil No.	Individual determinations.				Average.
	1	2	3	4	
A	60.5	60.8	61.8	63.0	61.5
B	62.2	62.3	62.5	63.7	62.7
C	72.9	73.4	73.1	74.7	73.5
D	61.5	62.3	63.4	61.9	62.2

In Table IV are reported the data on a series of 36 loess soils, including the maximum water capacity, the hygroscopic coefficient calculated from the preceding by the Briggs-Shantz formula, and the departure of the calculated from the determined value, previously reported (1, p. 216). The samples were collected from 30 virgin prairie fields in Nebraska, 5 near each of the 6 towns mentioned at the head of the columns. All were taken from fields classified by the Federal Bureau of Soils as "Marshall silt loam" or "Colby silt loam." In each field 10 borings were made to a depth of 6 feet and composite samples prepared of each foot section, thus giving 6 samples from each field. From these were prepared the samples used in this work, each of the latter being prepared by mixing equal weights of the corresponding five field samples (1, p. 204). Each value for the directly determined hygroscopic coefficients represents the average of 10 determinations (1, p. 214).

TABLE IV.—*Maximum water capacities of loess soils in Nebraska and the hygroscopic coefficients calculated from these by the Briggs-Shantz formula*

MAXIMUM WATER CAPACITY

Depth.	Wauneta.	McCook.	Holdrege.	Hastings.	Lincoln.	Weeping Water.	Average.
Foot.							
1	66.0	65.4	72.0	69.7	69.5	73.5	69.3
2	64.4	63.0	68.8	75.6	69.9	66.8	68.1
3	61.6	60.6	67.4	73.5	63.4	62.5	64.8
4	62.2	59.7	61.0	70.1	64.2	63.7	63.5
5	59.4	58.1	61.5	67.0	63.4	62.3	61.9
6	55.8	57.8	62.7	63.0	66.2	60.2	60.9
Average	61.6	60.8	65.6	69.8	66.1	64.8	64.8

CALCULATED HYGROSCOPIC COEFFICIENT

1	11.3	10.3	11.9	11.4	11.3	12.3	11.4
2	10.1	9.8	11.2	12.8	11.4	10.7	11.0
3	9.5	9.2	10.8	12.3	9.5	9.7	10.2
4	9.6	9.0	9.4	11.5	10.1	10.0	9.9
5	8.9	8.8	9.5	10.6	9.9	9.6	9.5
6	8.1	8.3	9.7	9.8	10.6	9.2	9.4
Average	9.6	9.2	10.4	11.4	10.5	10.2	10.2

DEPARTURE OF CALCULATED FROM DETERMINED HYGROSCOPIC COEFFICIENT

1	2.2	0.3	1.8	2.8	−0.7	0.2	1.1
2	.5	−1.1	.0	1.2	−3.0	−3.0	−.9
3	−.2	−1.5	−.5	−.1	−4.1	−4.2	−1.7
4	−.3	−.7	−.8	.4	−2.9	−3.0	−1.2
5	−.1	−.3	−.1	−.1	−2.9	−3.0	−1.1
6	−.2	−.8	.3	−.9	−2.1	−3.3	−1.2
Average	.3	−.7	.1	.5	−2.6	−2.8	−.8

In the case of rather more than half of the samples the calculated values agree satisfactorily with the directly determined hygroscopic coefficients. In the case of the Lincoln and Weeping Water samples, which are richest in clay and silt, and correspondingly poorest in very fine sand (2, p. 407), the calculated values are much too low for all except those samples from the surface foot. As an index of texture the calculated hygroscopic coefficients would be very misleading. For instance, the Wauneta samples, with an average of 9.6, would appear almost as fine-textured as the Weeping Water soils with an average of 10.2, whereas in fact actual mechanical analyses have shown the latter to contain nearly three times as much clay, more than half as much again silt, and only about one-fourth as much very fine sand (Table V). It is of interest that in the case of those members of this series with which satisfactory results are obtained by the Briggs-Shantz formula for the calculation of the hygroscopic coefficient from the mechanical analysis the formula of the same authors for the calculation of this value from the maximum water capacity fails, and vice versa.

TABLE V.—*Relation of calculated hygroscopic coefficients to the texture of Nebraska soils.*[a]

Item.	Wauneta.	McCook.	Holdrege.	Hastings.	Lincoln.	Weeping Water.
Maximum water capacity	*Per cent.* 61.6	*Per cent.* 60.8	*Per cent.* 65.6	*Per cent.* 69.8	*Per cent.* 66.1	*Per cent.* 64.8
Calculated hygroscopic coefficient	9.6	9.2	10.4	11.4	10.5	10.2
Determined hygroscopic coefficient	9.2	9.9	10.3	11.0	13.1	13.0
Gravel, above 1.0 mm.	.0	.0	.0	.0	.1	.0
Coarse sand, 1.0—0.5 mm.	.1	.1	.2	.2	.4	.2
Medium sand, 0.5—0.25 mm.	.3	.4	.3	.5	.7	.3
Fine sand, 0.25—0.10 mm.	1.9	1.5	1.3	1.9	2.4	1.2
Very fine sand, 0.10—0.05 mm.	49.3	38.6	27.9	21.6	10.3	12.7
Silt, 0.05—0.005 mm.	42.2	51.6	62.6	64.5	68.5	68.5
Clay, 0.005—0.000 mm.	6.3	7.8	7.7	11.3	17.6	17.3

[a] The mechanical analyses are from Alway and Rost (2, p. 407.)

The series of samples reported in Table VI includes, in addition to loess soils from Nebraska, residual soils from the same State and a few samples from the Southwestern States. The data upon both the hygroscopic coefficient and the water capacity are the means of five or more concordant determinations. The range in texture is much wider than that of those in Table IV and quite similar to that of the soils dealt with by Briggs and Shantz (4, p. 67). Except in a few cases, the calculated value agrees fairly well with the directly determined value. The widest departures are shown by the eastern Nebraska loess subsoil, similar in texture to the Lincoln and Weeping Water subsoils reported in Table IV and by two surface soils,

one from New Mexico and the other from Arizona. Three residual soils from Nebraska, No. 4, 5, and 6, which showed very similar hygroscopic coefficients, differ distinctly in water capacity.

TABLE VI.—*Comparison of the determined hygroscopic coefficient with that calculated from the maximum water capacity*

Soil No.	Description of soil.	Maximum water capacity.[a]	Hygroscopic coefficient.		
			Directly determined.[b]	Calculated from maximum water capacity.[c]	Departure of calculated coefficient from that found.
A	Dune sand, western Nebraska.....	25.8	0.6	1.1	0.5
1	Desert sand, Palm Springs, Cal.....	28.9	.9	1.9	1.0
2	Sandy subsoil, Palm Springs, Cal.....	27.0	1.1	1.4	.3
3	Desert sand, Orogrande, N. Mex.....	27.1	1.7	1.4	— .3
4	Sandy surface soil, western Nebraska..	34.2	3.3	3.1	— .2
5	Sandy subsoil, A, western Nebraska...	31.0	3.4	2.3	—1.1
6	Sandy subsoil, B, western Nebraska...	36.0	3.4	3.5	.1
7	Sandy loam subsoil, western Nebraska.	46.3	5.6	5.9	.3
8	Sandy loam surface, western Nebraska.	53.4	7.1	7.6	.5
9	Silt loam subsoil, A, western Nebraska.	57.2	7.6	8.5	.9
10	Silt loam subsoil, B, western Nebraska.	55.4	8.2	8.1	— .1
11	Red loam surface, Cuervo, N. Mex....	49.0	10.0	6.6	—3.4
12	Silt loam surface, A, western Nebraska.	56.8	10.1	8.4	—1.7
13	Silt loam surface, eastern Nebraska....	60.9	10.2	9.7	— .5
14	Silt loam surface, B, western Nebraska.	63.7	10.5	10.0	— .5
15	Adobe surface soil, McNeal, Ariz.....	60.3	12.9	9.0	—3.9
16	Silt loam subsoil, eastern Nebraska....	65.7	13.3	10.5	—2.8

[a] Determined by Mr. J. C. Russel.
[b] Determined by Mr. G. R. McDole.
[c] Using Briggs-Shantz formula: Hygroscopic coefficient=(maximum water capacity−21)×0.234.

Thus, while the Briggs-Shantz formula with many soils gives values fully in accord with those directly determined, with many others it gives results so widely divergent that it can not be regarded as sufficiently reliable for studies of available soil moisture, or even for soil-survey purposes.

ESTIMATION OF THE HYGROSCOPIC COEFFICIENT FROM THE HYGROSCOPIC MOISTURE

The hygroscopic coefficient indicates the maximum of hygroscopic moisture, the amount found when a more or less completely dried soil has been kept in contact with a saturated atmosphere at a constant temperature until the moisture in the soil is in approximate equilibrium with that in the atmosphere. Theoretically, actual equilibrium would not be attained until the moisture content of the soil equaled that of the same soil in actual contact with water (13, p. 448), but the time required for this is so great that this theoretical consideration does not affect the present discussion.

If two soils, A and B, be allowed in one case to reach equilibrium with a saturated atmosphere and the hygroscopic coefficients thus found be *a* and *b*, respectively, and in another case the same soils be allowed to reach

equilibrium with a partially saturated atmosphere, the amounts of hygroscopic water present in the latter case, a' and b', should bear the same relation to one another as do the hygroscopic coefficients—

$$\frac{b}{b'} = \frac{a}{a'} \text{ or } b = a\,\frac{b'}{a'}$$

If the soils can conveniently be brought into equilibrium with the partially saturated atmosphere, it would simply be necessary to determine accurately the hygroscopic coefficients of a few typical soils of which large quantities of thoroughly mixed, air-dried samples have been prepared and then expose to a partially saturated atmosphere portions of some of these along with the samples of which the hygroscopic coefficients are desired. From the found amounts of hygroscopic moisture the hygroscopic coefficients could be calculated by the above formula. This would obviate many of the inconveniences connected with the determination of hygroscopic coefficients, including the difficulty of obtaining a fully saturated atmosphere and of preventing dew formation through fluctuations of temperature.

As we had a series of foot samples of loess which had already been subjected to careful hygroscopic-coefficient determinations (1, p. 215–216), they were the first to be tried. The samples, after being brought from the fields in cloth sacks, had been stored for several months in the unheated but well-ventilated attic of the Nebraska Experiment Station building and later reduced to the desired degree of fineness, thoroughly mixed, placed in sealed jars, and again stored in the attic. About a year previous the moisture had been determined in some 50 of the field samples before preparing the composites and had been found to lie between the limits of 2.5 and 4.9, practically the same as found in this experiment. In shallow aluminum trays 5 by 7 inches with edges 0.75 inch high the samples were exposed in triplicate on the shelves of the attic storeroom mentioned above. Each tray carried about 10 gm. of soil. All the samples were exposed for seven days, those from Wauneta and McCook from March 15 to 22 and the others from March 25 to April 1. With the first set the range of temperature in the air of the room was 1° to 14° C.; and that of the humidity at the Lincoln (Nebr.) station of the Weather Bureau, 2 miles distant, was 67 to 94 per cent, while with the second the corresponding data were 9° to 23° C. and 42 to 93 per cent. Three of the samples which had been exposed in the first set were exposed again with the second and were found to have the same amount of hygroscopic moisture in both cases. Accordingly we may assume that all of the former contained the same amount of moisture that they would have shown if exposed with the second set, with which there were exposed in duplicate two soils, H and S, which had been repeatedly used as control soils in the determination of hygroscopic coefficients and for which, accordingly, we had a great many concordant determinations, the average of which was 5.6 for H and 22 for S. The

hygroscopic moisture is reported in Table VII. The triplicate determinations were concordant, in most instances differing by less than 0.2 per cent. The ratio of the hygroscopic coefficient to the found hygroscopic moisture (Table VII) shows an average of 2.71 and, with the exception of the first foot at Hastings and the fourth at Wauneta, lies between 2.38 and 2.96, a range of less than 25 per cent.

TABLE VII.—*Hygroscopic moisture in a series of air-dried loess soils and its relation to the hygroscopic coefficient in these soils*

HYGROSCOPIC MOISTURE

Depth.	Wauneta.	McCook.	Holdredge.	Hastings.	Lincoln.	Weeping Water.	Average.
Foot.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
1	3.1	3.5	3.8	3.0	4.2	4.1	3.6
2	3.4	4.0	4.7	4.2	5.5	4.9	4.4
3	3.4	3.7	4.7	4.6	5.4	5.0	4.4
4	3.3	3.4	4.3	4.3	5.0	4.9	4.2
5	3.2	3.2	4.0	4.1	4.9	4.8	4.0
6	2.8	3.2	3.8	4.2	4.8	4.7	3.9
Average	3.2	3.5	4.2	4.1	5.0	4.7	4.1

RATIO OF HYGROSCOPIC COEFFICIENT TO HYGROSCOPIC MOISTURE

1	2.93	2.86	2.66	3.20	2.86	2.95	2.91
2	2.82	2.72	2.38	2.76	2.62	2.80	2.68
3	2.85	2.89	2.40	2.70	2.52	2.78	2.69
4	3.00	2.85	2.38	2.58	2.60	2.65	2.68
5	2.81	2.93	2.40	2.61	2.61	2.62	2.66
6	2.96	2.84	2.47	2.60	2.64	2.66	2.69
Average	2.91	2.80	2.45	2.70	2.62	2.76	2.71

CALCULATED HYGROSCOPIC COEFFICIENT *a*

1	8.4	9.5	10.3	8.1	11.4	11.1	9.8
2	9.2	10.8	12.7	11.4	14.9	13.3	12.0
3	9.2	10.0	12.7	12.5	14.6	13.6	12.1
4	8.9	9.2	11.7	11.7	13.6	13.3	11.4
5	8.7	8.7	10.8	11.1	13.3	13.0	10.9
6	7.3	8.7	10.3	11.4	13.0	12.7	10.6
Average	8.7	9.5	11.4	11.1	13.6	12.7	11.2

DEPARTURE OF CALCULATED FROM DETERMINED HYGROSCOPIC COEFFICIENT

1	−0.7	−0.2	0.2	−1.5	−0.6	−1.0	−0.7
2	− .4	− .1	1.5	− .2	.5	− .4	.1
3	− .5	− .7	1.4	.1	1.0	.4	.2
4	−1.0	− .5	1.5	.6	.6	− .3	.3
5	− .3	− .4	1.2	.4	.5	− .4	.3
6	−1.0	− .4	.9	.7	.4	− .2	.1
Average	− .6	− .4	1.1	.1	.5	− .3	.1

[a] Using ratio found with control soils H and S.

The control soils H and S, exposed in the second set, contained 2.3 and 7.4 per cent of hygroscopic moisture, respectively, thus giving ratios of 2.44 and 2.98, respectively, with an average of 2.71. The hygroscopic coefficients calculated from the hygroscopic moisture, using this average ratio, and the departures of these from the directly determined values are reported in Table VII. In general, the departure is slight and on the average is negligible. However, it should be pointed out that the latter circumstance is due to the average of the ratios for the two control soils being the same as that for those of the 36 samples under consideration. If we had used only H as a control, the calculated values would have been one-tenth higher, while if S alone had been employed, they would have been one-tenth lower. In either of these two cases, however, the calculated values would have been much nearer the directly determined hygroscopic coefficients than would the values computed from the moisture equivalents by the Briggs-Shantz formula (3, p. 839).

We similarly exposed several other series of soils of which the hygroscopic coefficients had already been determined. The concordance of the calculated with the found values was much alike in all. The first of these series, which was strictly typical, is reported in Table VIII. It consisted of 24 samples, part surface soils and part subsoils, but all derived from residual material in western Nebraska. The soils were exposed on metal trays on the shelves of an inclosed basement room for two weeks, April 29 to May 13. The maximum temperature recorded in the room during this period was 22° C., and the minimum 18°; observations in this room, extending over three years, had shown that there was but rarely a daily range exceeding one degree. In the table the soils are arranged in order of texture. The ratio for the two control soils exposed in triplicate was 2.9. The data on the determined hygroscopic coefficients are the means of duplicate determinations. The calculated values in nearly all cases agree satisfactorily with those directly determined. The greatest divergences are shown by soils 13 and 23, the calculated hygroscopic coefficient being one-sixth too low for the former and one-seventh too high for the latter.

Finally we exposed on paper pie plates 145 soils of which the hygroscopic coefficients had not been determined. Each soil was exposed in duplicate and there were also 15 plates of each of two control soils, La and S. In the case of both of the control soils triplicate samples were exposed in different parts of the room in order to determine whether the position in the small room exerted a marked influence upon the amount of hygroscopic moisture absorbed. During the eight days of exposure, June 5 to 13, the temperature ranged from 18° to 23° C.

The position in the room was found to have a slight but appreciable influence upon the amount of moisture absorbed, the extremes being shown by two shelves, on one of which the samples of S and La were found to have 9.3 and 3.8 per cent, respectively, and on the other 8.7

and 3.3. The averages for the 15 samples of each were 8.9 for S and 3.5 for La. The ratio was accordingly 2.47 for S and 2.74 for La, the hygroscopic coefficients being 22.0 and 9.6 per cent, respectively.

TABLE VIII.—*Comparison of the determined hygroscopic coefficients with those computed from the hygroscopic moisture in a series of residual soils from southwestern Nebraska*

Soil.	Hygroscopic moisture.	Hygroscopic coefficient.			Ratio of hygroscopic coefficient to hygroscopic moisture.
		Determined.	Computed.	Departure.	
	Per cent.				
H (control)	1.9	5.6	.....	.....	2.9
S (control)	3.5	10.2	.....	.....	2.9
1	.3	.8	0.9	0.1	2.7
2	.3	.8	.9	.1	2.7
3	.3	.8	.9	.1	2.7
4	.3	.9	.9	.0	3.0
5	.4	1.1	1.2	.1	2.8
6	.4	1.1	1.2	.1	2.8
7	.4	1.4	1.2	−.2	3.5
8	.5	1.4	1.4	.0	2.8
9	.7	2.2	2.0	−.2	3.1
10	.8	2.2	2.3	.1	2.8
11	1.7	4.4	4.9	.5	2.6
12	1.3	4.5	3.8	−.7	3.5
13	1.8	5.4	5.2	−.2	3.0
14	1.7	5.9	4.9	−1.0	3.5
15	2.4	6.6	7.0	.4	2.7
16	2.5	8.0	7.2	−.8	3.2
17	2.9	8.3	8.4	.1	2.8
18	2.7	8.4	7.8	−.6	3.1
19	3.5	9.7	10.1	.4	2.8
20	3.6	9.8	10.4	.6	2.7
21	3.6	10.7	10.4	−.3	3.0
22	3.6	10.8	10.4	−.4	3.0
23	5.7	14.4	16.5	2.1	2.6
24	5.2	14.9	15.1	.2	2.9

At this point the removal of the junior author to California interrupted the work, but during the following year Mr. A. Skudrna made single determinations of the hygroscopic coefficients in the usual manner, using also the same control samples. In Table IX are shown the ratios of typical sets for various fields, the others being similar. It will be seen that the ratio is not affected by the presence of organic matter, as in the surface 6 inches it is similar to that in the subsoil.

This set serves well to illustrate the advantage of the indirect method of determination. During the early part of May the samples had been collected from 23 fields in western Nebraska for the study of field moisture conditions, which happened to be of unusual interest at that time, a wet winter and early spring having succeeded an exceptionally dry summer and autumn. The moisture samples had been weighed into light cotton sacks and dried at 110° C. to constant weight, and then left exposed to the air for a week or two before being placed on the plates in the so-called "constant temperature room." By the middle of June, as it later proved, reliable data on the unusual field moisture conditions

were at hand. The ratio varied in the case of the individual soil samples from 2.0 to 2.7, and the average ratio for the different fields only from 2.1 to 2.6. While the ratio tended to be higher in the coarse-textured soils there were many exceptions to this generalization.

TABLE IX.—*Ratio of hygroscopic coefficient to hygroscopic water in air-dried soils from typical fields in western Nebraska, May, 1912*

Depth.	Prairies.			Corn stubble.			Winter wheat.		Average of all.
	I.	II.	III.	IV.	V.	VI.	VII.	VIII.	
Foot.									
0–½	2.7	2.4	2.5	2.3	2.5	2.2	2.2	2.1	2.4
½–1	2.7	2.4	2.6	2.5	2.6	2.2	2.2	2.1	2.4
2	2.3	2.4	2.7	2.1	2.4	2.4	2.0	2.0	2.4
3	2.7	2.3	2.6	2.1	2.4	2.2	2.2	2.2	2.3
4	2.5	2.2	2.5	2.2	2.1	2.4	2.2	2.1	2.3
5	2.6	2.2	2.6	.....	2.0	2.3	2.1	2.3	2.3
6	2.6	2.1	2.3	2.3	2.5	2.6	2.1	2.0	2.3
Average	2.6	2.3	2.5	2.3	2.4	2.3	2.1	2.1	2.3

Table X and figure 1 show the concordance of the data on free water, using in the one case the computed and in the other the directly determined hygroscopic coefficients. Whichever set, B or C, is used, the same general moisture relations are shown, and for the purposes of the

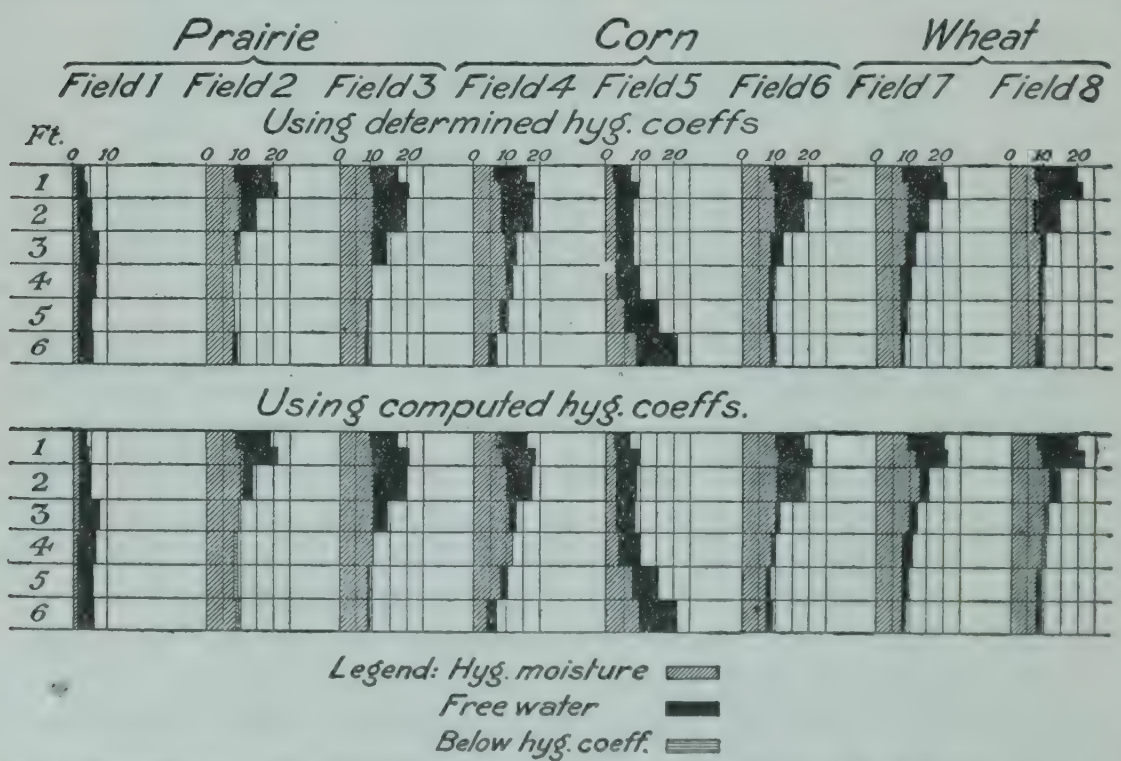

FIG. 1.—Diagram showing the amounts of free water at different levels in eight fields, illustrating the concordance of the values obtained for the hygroscopic coefficient by calculation from the hygroscopic moisture with those directly determined.

field moisture study they are almost equally satisfactory, but the data in C were all at hand a month after the field work had been completed, while those in D were not obtainable within half a year without dealing with this particular series out of its regular order.

TABLE X.—*Moisture conditions in typical fields of western Nebraska in May, 1912, showing the applicability of the data obtained by computing the hygroscopic coefficient from the hygroscopic water in the air-dried soil*

A.—TOTAL WATER IN SOIL IN FIELD

Depth.	Prairies.			Corn stubble.			Winter wheat.	
	I	II	III	IV	V	VI	VII	VIII
Foot.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
0–½	3.6	19.4	17.1	16.3	7.3	18.9	20.4	20.1
½–1	4.5	21.6	20.2	18.7	10.0	21.6	21.5	22.0
2	5.4	14.3	19.9	17.8	8.7	19.3	16.0	15.1
3	7.9	9.4	14.3	13.1	8.5	13.8	12.3	10.5
4	6.9	8.0	9.6	12.0	10.0	9.8	10.7	9.6
5	5.9	8.1	9.1	10.5	15.6	9.2	9.4	9.4
6	6.1	8.8	9.1	6.9	21.3	9.4	8.8	9.6
Average [a]	6.0	11.5	13.4	13.0	12.1	13.6	13.0	12.5

B.—FREE WATER (USING DETERMINED HYGROSCOPIC COEFFICIENTS)

0–½	2.0	10.8	8.2	10.4	5.3	10.4	12.2	12.8
½–1	2.9	11.0	10.7	10.5	7.1	11.5	11.2	13.4
2	3.8	4.0	9.5	9.5	6.1	9.1	6.2	7.3
3	6.0	1.1	4.3	3.7	5.9	5.3	3.9	1.1
4	5.4	.5	.0	2.0	7.0	1.7	2.9	.9
5	4.6	.3	.7	2.7	10.0	1.5	1.9	.8
6	4.8	1.2	1.5	2.4	12.4	.9	1.4	2.1
Average	4.2	3.0	4.0	5.1	7.9	4.9	4.7	4.2

C.—FREE WATER (USING COMPUTED HYGROSCOPIC COEFFICIENTS)

0–½	2.1	10.5	7.9	9.7	5.3	9.0	10.7	12.6
½–1	3.0	10.2	10.7	10.3	7.2	10.1	9.8	12.8
2	3.6	3.1	10.2	7.8	5.9	8.6	3.1	3.6
3	6.1	.0	4.6	1.9	5.7	4.1	2.4	−.4
4	5.4	−.7	−.1	.3	6.4	1.1	2.7	−.8
5	4.6	−.8	.7	.....	8.3	1.3	1.4	1.6
6	4.8	−.3	.7	2.6	12.1	1.5	1.0	1.8
Average	4.2	1.9	4.2	[b] 4.2	7.4	4.4	3.5	3.1

[a] In estimating both the average hygroscopic coefficient and the average moisture content of the 6 feet of soil we have employed the mean for the first foot instead of averaging the seven data in each column.
[b] In estimating the average the missing datum is replaced by that from the preceding part of the table.

It should be pointed out that in the case of all the soils with which we have employed the simpler method so satisfactorily the samples either had been dried at 110° C. or for many months had been exposed to the dry air of the storeroom and so had attained an air-dried condition. As it would in many instances be more convenient if the moist samples could be exposed on paper trays or plates and left until they came into equilibrium with the atmosphere, we exposed for varying periods—2 to 21 days—10 to 15 gm. samples of the control soils, H, La, and S, all containing the maximum hygroscopic moisture, together

with samples of the same soils dried at 100° C. and still others that were in an air-dried condition. At the end of from 3 to 7 days those of the second and third sets were alike in moisture content, while those of the first were distinctly moister. A difference was frequently shown even after an exposure of 21 days.

Using 5-, 10-, 15-, and 20-gm. samples of the same three control soils we compared paper with aluminum trays. Both gave the same results with H and La, but in some cases S, which had a coefficient of 22.0, gave a somewhat lower result on the paper trays, 7.7 as compared with 8.0, as though, following an increase in the relative humidity of the air, the material of the trays, being in itself hygroscopic, had competed with the contained soil for moisture from the atmosphere.

From the above statements it would appear that once having accurately determined the hygroscopic coefficient of suitable control soils it would only be necessary to expose for a few weeks on trays in a closed room thin layers of the air-dried or oven-dried soils under investigation, together with a sufficient number of trays containing the control soils. After sufficient exposure the mere determination of the hygroscopic moisture in all would permit of the calculation of the hygroscopic coefficients. Thus, the hundreds or thousands of samples which might be collected during the summer in connection with a soil survey might be placed in trays on the shelves as they reached the laboratory, the drying being left until winter. There could then be obtained that single-valued factor expressing texture which appears to us the most desirable of all those so far proposed (1, p. 214).

In connection with the method there remains to be determined, among other things, the minimum time of exposure necessary, the most suitable material for trays, the desirability of providing for the agitation of the air in the exposure room and maintaining the humidity of the air in the room between definite limits.

This indirect method of estimating the hygroscopic coefficient appears to give more reliable results than those to be obtained by the use of a single formula applied to either the mechanical composition (2, p. 411) or the moisture equivalent (3, p. 842), while at the same time requiring only the simplest equipment, as well as a minimum of skill on the part of the operator, and being economical of time.

SUMMARY

The estimation of the hygroscopic coefficient from the maximum water capacity, while with many soils giving values in full accord with those directly determined, with so many soils gives such erroneous results that it is to be regarded as too unreliable for use in connection with either studies of available soil moisture or for soil-survey purposes.

From the studies reported it appears that the hygroscopic coefficient may be calculated from the hygroscopic moisture found in a soil which

has been allowed to come into equilibrium with an only partially saturated atmosphere and that this method will require only simple equipment, a minimum of skill on the part of the operator, and be so economical of time as to recommend it wherever a very large number of samples have to be dealt with.

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CORRELATION BETWEEN THE SIZE OF CANNON BONE IN THE OFFSPRING AND THE AGE OF THE PARENTS [1]

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Jensen (3) [3] and the writer (12, 13) have both found that in the Jutland and Gudbrandsdal horses the young and relatively young animals have given the best offspring and that old animals have very seldom produced high-class stallions. Both of these breeds are selected for heavy bones. On the other hand, in the English thoroughbred, which is selected for speed, old horses produce high-class animals for that purpose. It is, then, naturally suggested that an investigation be made as to whether young parents are producing heavier cannon bones than old parents. Von Oettingen (7) noticed this in numerous cases and stated that the offspring of the halfbred stallions Halm, Harnish, and Optimus (in Gudwallen and Trakehnen, Germany), with relatively heavy bones, tended to become slender in the cannon bones when the stallions became old. Jensen made a similar statement in a letter to the author about the offspring of the famous Jutland stallion Aldrup Munkedal. The writer has also had the same experience with Gudbrandsdal stallions. In Percheron horses bred in France, where young stallions and mares are much used for breeding purposes, it is recognized that the breed shows a big increase in cannon-bone size. This can not be substantiated by measurement, but it seems to be a fact. Of statements concerning other mammals that show a similar tendency, it is worth while to cite Stonehenge's (11) observation concerning dogs: "When, however, the produce is desired to be very small, the older both animals are the more likely this result is."

When these views are considered, although they are not supported by any scientific measurements, there seems to be good reason for undertaking an investigation of the correlation between the age of the parents and the measurements of the cannon bone of the offspring. Fortunately the Gudbrandsdal stud book [4] contains material that is suitable for such an investigation. The writer selected as material for the investigation all mares measured in the "South of Norway" district

[1] Contribution from the Laboratory of Genetics, Agricultural Experiment Station of the University of Illinois.

[2] The author wishes to express his thanks to Prof. J. A. Detlefsen and Prof. H. L. Rietz, of the Illinois Station, for many suggestions and criticisms in connection with this investigation.

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(Söndenfjeldske). These mares are all measured in the same way, and the measurements are registered in the stud book. The measurement is taken near the middle of the cannon bone, at its narrowest point. In the data the writer found five mares whose dams were 1 year old at the time of service. Both because this number is too small to give average values whose significance can be estimated, and because of the abnormal character of these extremely young dams, they have been excluded in the general treatment of the problem. Two other mares are excluded, one for the reason that the writer knows that her cannon bone is abnormally thickened, and the other because the measurement of the side breadth is abnormally small compared with the circumference.

Along with age, there are other causes that have an influence on the size of the cannon bone of the offspring. Heredity is the first of these causes to be considered, because it is probably of great importance in this particular investigation, since the modern Gudbrandsdal horses include blood lines that represent a cross between the original horse type of eastern Norway (the most northern branch of the occidental horse) and different light-horse types. In the seventeenth and eighteenth centuries there were imported into Norway stallions of the Fredricsborgian horse or a closely related type. The Fredricsborgian horse was a light-horse type that descended from crosses of Seeland country horses, Spanish, and oriental horses. In the nineteenth century there was imported into Norway an English thoroughbred stallion, Odin. This stallion became by chance the founder of one of the most important stallion lines of the Gudbrandsdal breed. In the nineteenth century the blood of the other Norwegian breed, the Fjord horse, was mixed with the Gudbrandsdal horse by the use of several mares, and thus became an influence on the latter breed (12, 13). If cannon-bone size is inherited, it is perfectly clear that the Gudbrandsdal horse is not a pure-bred in a genetic sense, since extreme heterozygosis must result from the numerous infusions of blood from such diverse sources.

One should consider the influence of nourishment on the size of the cannon bone, but the number of individuals in this investigation is so large and the conditions of nourishment are such that the effects of different nourishments would probably eliminate each other, or at least would be insignificant. Although nutrition undoubtedly has a pronounced effect upon size and growth, the writer has assumed that under good conditions the adult size of the cannon bone represents in a fair way the inherent possibilities of an animal. Such has been the usual assumption in genetic investigations of size characters.

The first task was to calculate the correlation between the age of the sires and the size of the cannon bone of female offspring. Table I gives the number and kinds of mares sired by stallions of different ages. Measurements under 18 cm. and over 21 cm. are arranged in the subclass headed 18 and 21, respectively. The averages of arrays

are calculated from the original figures before they were grouped in a correlation table. The age of the stallions is arranged in classes with 3-year intervals in each class—that is, class 1 is composed of stallions 2 to 4 years old; class 2 is composed of stallions 5 to 7 years old, etc. In this notation 2 to 4 means under 5, 5 to 7 means under 8, and so on. The correlation coefficient is -0.061 ± 0.012. Although the coefficient of correlation is five times as large as the probable error, the negative correlation is so small that it indicates only a very slight tendency for older sires to beget daughters with smaller cannon bones. The average size of cannon bone decreases slightly as the age of the sires increases. The difference between size of cannon bone for the mares whose sires are 5 to 7 years old and the mares whose sires are 17 years of age or older is 0.111 ± 0.036.

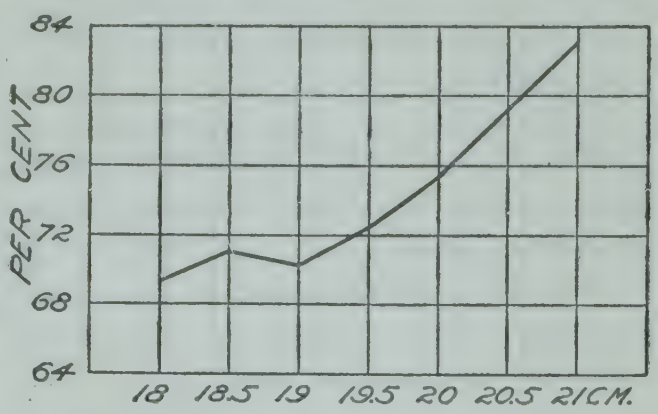

FIG. 1.—Curve showing the percentages of mares with various-sized cannon bones, sired by stallions under 11 years old.

TABLE I.—*Correlation between the age of the sires and the measurement of the cannon bones of the female offspring*

Age of stallion.	Number of female offspring having a cannon bone of a given size (cm.).								Average size of cannon bone.
	18	18.5	19	19.5	20	20.5	21	Total.	
Years.									*Cm.*
2 to 4	26	58	143	96	72	14	6	415	19.273±0.021
5 to 7	73	137	387	182	178	44	22	1,023	19.272±0.014
8 to 10	60	99	252	107	135	22	16	691	19.244±0.017
11 to 13	33	61	166	75	73	11	7	426	10.219±0.021
14 to 16	24	37	102	47	37	7	2	256	19.175±0.026
17 and older	10	23	58	26	20	3	0	140	19.161±0.033
Total	226	415	1,108	533	515	101	53	2,951	

$r = -0.061 \pm 0.012$

Figure 1 shows the percentages of all mares of various size classes sired by stallions 10 years and younger. To be sure, more mares of all classes were sired by stallions under 11 years old, but it is interesting to note that gradually increasing percentages of the larger mares were sired by these younger stallions. To state this differently, mares whose cannon bone measures more than 19.5 cm. are more likely to come from sires under 11 years of age than are the mares whose cannon bone measures under 19.5 cm. There is a perceptible rise in the curve beginning at the 19.5 cm. Figure 1 was plotted from Table I by taking the ratio of the

number of mares in any given class whose sires were under 11 years to the total number of mares in that class. It would seem from the curve that a decided negative correlation existed, but one must remember that rapid rise in the right-hand part of the curve (20 cm. and above) is based upon 669 cases, whereas the left-hand portion is based upon 2,281 cases.

To get sufficiently large numbers and as homogeneous material as possible, the author has taken out for investigation daughters of five Government and five association stallions, each of which has more than 25 registered daughters, of which at least 10 are sired before or after the stallion was 10 years old (Table II). The following observations may be made from the table in regard to the average size of cannon bone for the daughters of these stallions.

TABLE II.—*Average size (in centimeters) of cannon bone in the daughters of 10 selected males, grouped according to the age of the males*

Name of sire.	Age of sire.	Total number of daughters.	Number of daughters having a cannon bone of a given size (cm.).							Average.	Difference.
			18	18.5	19	19.5	20	20.5	21		
Digre 222....	10 years and younger....	13	0	1	7	2	2	1	0	19.33 ±0.099	0.24±0.12
	Older than 10 years.....	25	3	3	13	2	3	0	1	19.09 ±0.074	
Bamsen 254..	10 years and younger....	13	0	0	7	0	6	0	0	19.52 ±0.085	0.41±0.12
	Older than 10 years.....	22	2	3	10	4	2	1	0	19.11 ±0.082	
Dölen 260....	10 years and younger....	11	1	2	1	2	5	0	0	19.39 ±0.14	0.39±0.16
	Older than 10 years.....	18	2	5	5	4	2	0	0	19.00 ±0.092	
Sverre 270...	10 years and younger....	10	0	0	7	0	3	0	0	19.30 ±0.096	0.06±0.11
	Older than 10 years.....	36	1	7	15	5	6	2	0	19.24 ±0.058	
Sindre 297...	10 years and younger....	36	3	2	13	8	9	1	0	19.35 ±0.058	0.11±0.10
	Older than 10 years.....	31	4	4	8	8	5	1	1	19.24 ±0.07	
Bjarne 301...	10 years and younger....	16	0	3	4	1	6	1	1	19.55 ±0.13	0.52±0.15
	Older than 10 years.....	29	2	6	14	4	3	0	0	19.03 ±0.068	
Galde 372....	10 years and younger....	60	3	4	19	9	20	3	2	19.49 ±0.061	−0.03±0.083
	Older than 10 years.....	37	1	1	11	10	14	0	0	19.52 ±0.057	
Kongen 376..	10 years and younger....	25	5	2	8	3	5	2	0	19.17 ±0.11	−0.21±0.16
	Older than 10 years.....	13	0	0	8	2	2	0	1	19.38 ±0.11	
Gimle 425....	10 years and younger....	69	2	5	20	19	17	3	3	19.51 ±0.051	0.90±0.09
	Older than 10 years.....	38	2	5	6	12	10	2	1	19.51 ±0.074	
Dalegudbrand 466..	10 years and younger....	47	3	3	14	11	5	5	6	19.57 ±0.086	0.19±0.11
	Older than 10 years.....	13	0	0	6	5	2	0	0	19.38 ±0.073	
Total..	10 years and younger....	300	17	22	100	55	78	16	12	19.448±0.026	0.216±0.037
	Older than 10 years.....	262		34	96	56	49	6	4	19.232±0.027	

Either the average for daughters sired when the stallion was 10 years old or younger, or the average when the stallion was older than 10 years, or the average in both these cases, is larger than the average for the breed.

Table II shows that for offspring of 7 of the 10 stallions the average size of the cannon bone is larger when the sire was 10 years old or younger at the time of service. The difference is not so large that it is significant in every case considered singly. The offspring of two, Galde and Gimle, of the remaining three sires show practically the same average for the two classes. The third stallion, Kongen, has a larger average for the daughters that he sired when he was older than 10 years. With such small numbers as 25 and 13 this deviation of this result from

our other results may be a chance fluctuation. In fact, the apparent lack of harmony of this case with the others is largely due to the size of the cannon bone of one mare—21 cm. This mare was sired when the stallion, Kongen, was 12 years old. The mother was 6 years old. With all 10 stallions it is remarkable how few there are of the daughters with a circumference of 20.5 and 21 cm. in that class which was sired when the stallions were older than 10 years. Taking the two classes as a whole, the writer finds for all daughters sired when the stallions were 10 years old and younger at time of service that the average cannon bone is 19.448 ± 0.026 and for the other class the average is 19.232 ± 0.027. The difference, 0.216 ± 0.037, is significant, for the difference is nearly 6 times the probable error. Figure 2 shows the percentage of the daughters of the 10 stallions that correspond to different sizes of cannon bone when the stallions were 10 years and younger at the time of service. Just as in the case of figure 1, there is a perceptible rise, the curve beginning at 19.5 cm. In other words, gradually increasing percentages of the larger mares (those over 19.5 cm.) were sired by these stallions when 10 years old or younger.

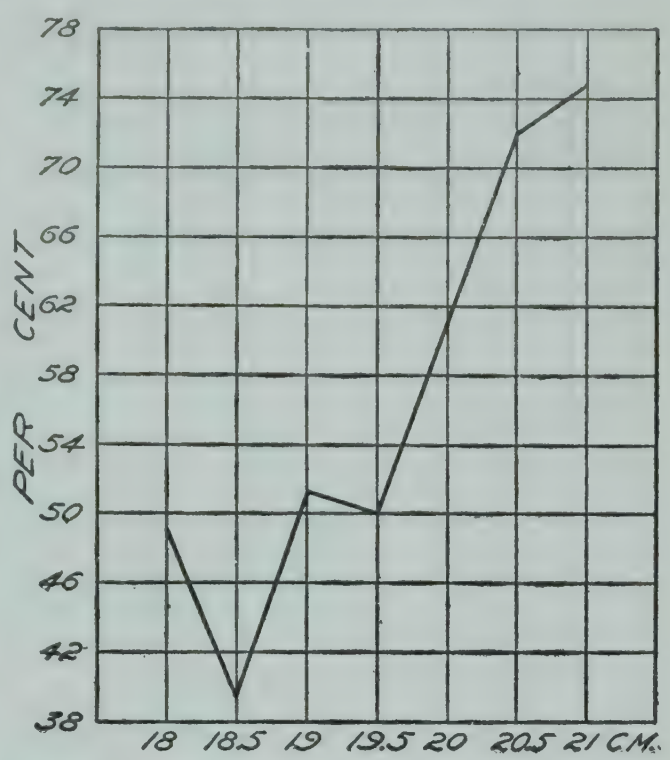

FIG. 2.—The percentages of mares with various-sized cannon bones, sired by 10 selected stallions when these were under 11 years old.

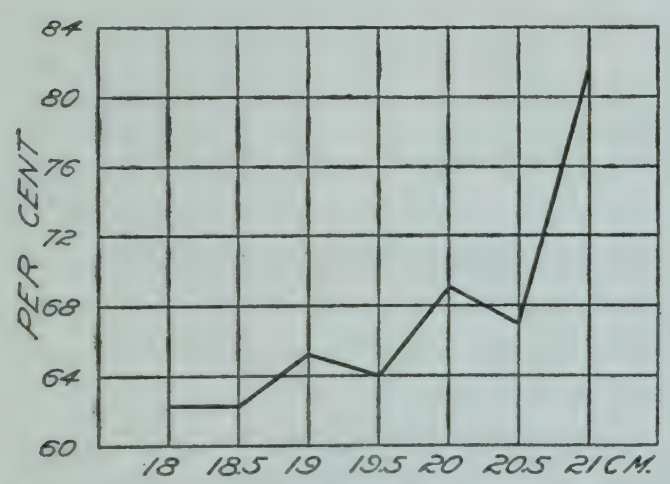

FIG. 3.—The percentages of mares of various-sized cannon bones bred from dams under 11 years old.

The correlation between the age of the dams and the size of the cannon bone of their female offspring was calculated to ascertain whether a similar relationship held as in the case of the sires in Table I. Curiously enough, the coefficient (-0.062 ± 0.017) is practically the same as that given in Table I. The total number of variates is not as large as that given in Table I, for the age of the dam is not as frequently registered

as that of the sire. Table III gives the number of mares bred by dams in the different age classes. The averages show that the young dams 2 to 4 years old have given offspring with the heaviest cannon bone. The difference between these and the class that has the lowest average is 0.159±0.039. The average for the class with dams 14 to 16 years old is 19.341±0.035. This average is practically the same as the average for the mares in the classes 5 to 7 and 8 to 10 year old class. This large average is due to the 41 mares with a circumference of 20 cm. Of these, 20 are sired by stallions 2 to 7 years old. Figure 3 shows the percentages of mares bred from dams 10 years old and younger. In this case also the rise begins in the neighborhood of 19.5 cm., and agrees with figures 1 and 2 in this respect.

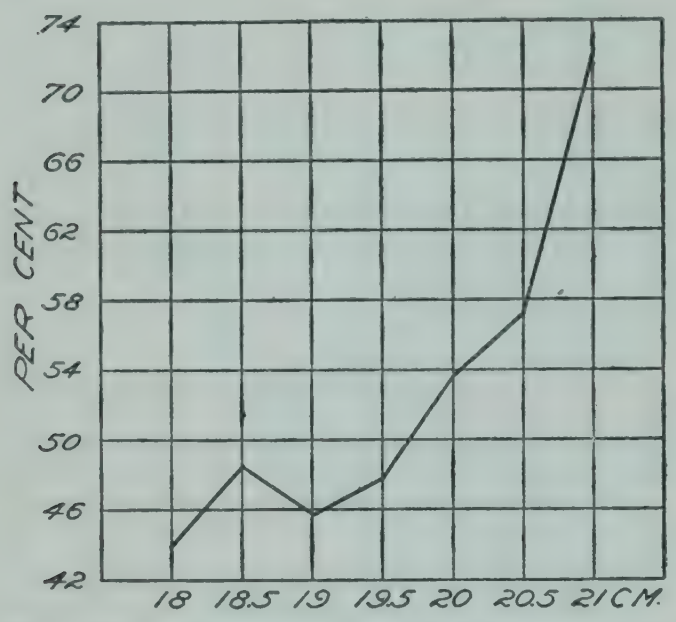

FIG. 4.—The percentages of mares of various-sized cannon bones bred from both parents under 11 years old.

TABLE III.—*Correlation between the age of the dams and the size of the cannon bone of the offspring*

Age of dam.	Number of offspring having a cannon bone of a given size (cm.).								Average size of cannon bone.
	18	18.5	19	19.5	20	20.5	21	Total.	
Years.									*Cm.*
2 to 4	13	34	80	65	60	15	9	276	19.404±0.028
5 to 7	21	48	155	87	87	17	12	427	19.344±0.023
8 to 10	22	32	177	70	66	18	7	332	19.340±0.025
11 to 13	14	37	103	56	32	14	4	260	19.245±0.027
14 to 16	13	16	57	33	41	9	2	171	19.341±0.035
17 and older	7	16	34	36	22	2	0	117	19.271±0.037
Total	90	183	546	347	308	75	34	1,583	

$r = -0.062 \pm 0.017$

In order to get a general view of the relation of the age of both parents to the size of the cannon bone of their offspring, the writer has computed in Table IV the average size of the cannon bone of the female offspring grouped according to whether both, either one, or neither of the parents was under 11 years of age. The differences between the averages in these groups are not striking, except when both parents are under 11 years old.

Figure 4 shows a rise beginning at 19.5 cm., which indicates that matings of parents both under 11 years old have given gradually increasing percentages of the larger size classes.

TABLE IV.—*Average size of the cannon bone in the females in relation to the age of both parents*

Age of parents.	Number of females having a cannon bone of a given size (cm.).								Average size of cannon bone.
	18	18.5	19	19.5	20	20.5	21	Total.	
									Cm.
Both parents 10 years old and younger	40	89	251	166	165	43	25	779	19.386±0.020
Sire 10 years old and younger, the dams older than 10 years	26	53	144	98	66	20	5	412	19.277±0.021
Sire older than 10 years, the dam 10 years old and younger	16	25	101	56	48	7	3	256	19.275±0.026
Both parents older than 10 years	8	16	50	27	29	5	1	136	19.291±0.036
Total	90	183	546	347	308	75	34	1,583	

TABLE V.—*Averages of the deviations of the daughters from their dams, grouped according to the age of both parents*

Age of parents.	Number of daughters having a given deviation in the size of the cannon bone (cm.).													Average deviation.
	−2.5	−2	−1.5	−1	−0.5	0	0.5	1	1.5	2	2.5	3	3.5	Total.	
															Cm.
Both parents 10 years old and younger	0	4	17	66	104.5	133	98.5	97.5	35	10.5	1	0	1	568	0.131±0.024
The sire 10 years old and younger. The dams older than 10 years	0.5	5.5	8.5	24.5	34	60.5	39	29	12.5	2.5	0.5	1	0	218	0.046±0.040
The sire older than 10 years. The dam 10 years old and younger	1	3	7	18.5	38	50	38	24	5	1.5	0	0	0	186	−0.016±0.039
Both parents older than 10 years	0	2	1.5	4	12.5	17	10.5	7	5.5	0	0	0	0	60	0.067±0.072

To show how the measurements of the cannon bones of the offspring deviate from those of the parents the writer has tabulated (Table V) the deviations of the daughters from the dams—that is, when the measurement of the cannon bone of the dam is 20 cm. and the cannon bone of the daughter is 19 cm., the daughter is entered in Table V under −1. In this table the mares that have both parents 10 years old and younger give an average deviation of 0.131 ±0.024 above the dam. This is 5½ times as large as the probable error. For the mares that have the sire 10 years old and younger, the dam being older than 10 years, and the mares with both parents older than 10 years, the difference is practically the same as the probable error. For the mares whose sires are older than 10 years and the dam younger, the difference is slightly negative

It is noteworthy that the daughters deviate from the dams by a significant amount only when both parents are 10 years old and younger, and in this case the daughters are larger than the dams in cannon-bone circumference. Figure 5 shows the percentages of mares in the various classes with both parents 10 years of age and younger. The curve was constructed from Table V. The offspring of parents under 11 years of age constitute about 55 per cent of the total number of individuals entered in the table, but the curve shows that the percentage of the daughters which average higher than their dams is greater than 55 per cent when both parents are under 11 years of age.

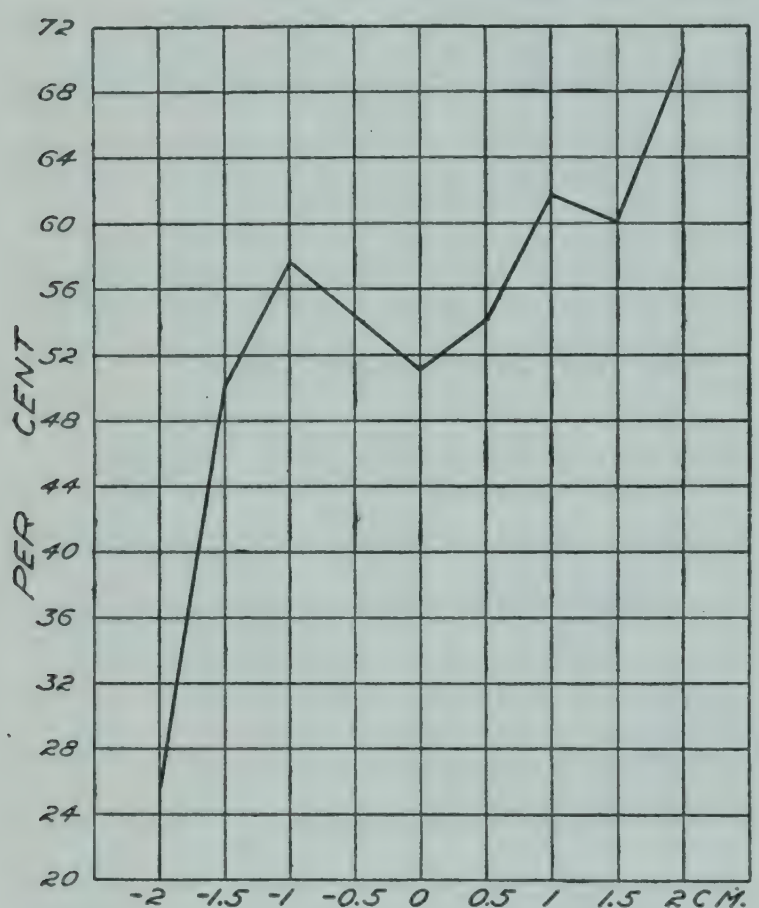

FIG. 5.—The percentages of mares in various classes deviating from their dams when both parents were under 11 years old.

It takes large numbers to establish the significance of the differences with which we are concerned in this paper, and it is to be hoped that further data will be obtained in order to test this matter for other heavy breeds. However, the present study points to the following conclusions:

(1) The age of the parent has an influence on the circumference of the cannon bone of the offspring.

(2) Immature parents 2 to 4 years old give offspring with the same measurement of the cannon bone as parents as old as 5 to 7 years.

(3) Parents older than 10 years considered as a class give offspring with lighter cannon bones than parents 10 years old and younger. In the breed examined there was found a larger percentage of individuals over average size whose parents were 10 years old or younger. On the other hand, the average individuals and those smaller have parents which are just as frequently under 10 years old as they are over. In other words, the lighter classes of cannon bone come as frequently from young as from old parents, but the heavier classes seem to come more frequently from younger parents.

(4) There seems to be some basis for the current opinion among breeders of Gudbrandsdal and other heavy breeds that young parents give better offspring than older parents.

It is extremely difficult to connect the data obtained with current genetic hypotheses and conceptions. The history of the Gudbrandsdal heavy horses leads one to expect heterozygosis. That the heavy registered mares and stallions should give a range of types is not surprising in itself, for such a recent breed can hardly be expected to breed true. However, in genetic investigations on size, segregation is supposed to be independent of age, and an increased proportion of large-sized offspring from the younger parents is hardly expected. It will naturally occur to the critical that circumstances entirely independent of heredity underlie these peculiar frequencies of the size classes. For example, one wonders whether more large offspring from young stallions and young mares are not registered in order to establish their reputation; or may not young stallions be mated to high-grade mares in order to make a better showing as sires, whereas this selection in mating would not be as rigorous after the sire had proven his worth? Many years of familiarity with this breed and with the methods of registration lead the writer to attach but little value to these considerations. The best mares and the best sires are those which are kept the longest for breeding purposes. The fact that measurements must accompany registration has prompted careful selection at all ages.

Available measurements or investigations that throw light on the inheritance of size in horses are scarce. It is not known whether cannon-bone size in horses is due to multiple factors such as are postulated in recent investigations on size in poultry by Punnett and Bailey (10), in rabbits by MacDowell (5), in ducks by Phillips (8, 9), in guinea pigs by Detlefsen (1), in corn by Emerson and East (2), and the like. The investigation of Landman (4) may throw some light on the inheritance of cannon-bone size in horses. East Prussian country mares, with relatively small cannon bones, were graded up by crossing them with Belgian stallions. Although the total numbers recorded are smaller than is desired as a basis for conclusions, they indicate the gradual rise in the hybrid animals approaching the average for Belgian mares (Table VI).

TABLE VI.—*Sizes of the cannon bone in the East Prussian country mares, Belgian mares, and three hybrid generations*

Animal.	Number of horses having a cannon bone of a given size (cm.).																	Average size of cannon bone.
	17	17.5	18	18.5	19	19.5	20	20.5	21	21.5	22	22.5	23	23.5	24	24.5	25	
East Prussian country mares	3	...	6	10	14	7	6	...	..	...	..	...	..	...	..	...	..	*Cm.* 18.83
F_1 generation	..	...	1	2	6	6	10	4	8	7	..	...	1	...	..	...	..	20.17
F_2 back cross	..	...	..	...	..	1	2	6	6	12	6	1	2	...	1	...	..	21.38
F_3 back cross	..	...	..	...	..	...	..	...	3	1	1	3	1	...	1	...	..	22.10
Belgian mares	..	...	..	...	..	...	..	1	..	1	..	2	2	1	4	2	3	23.53

The measurements for the Belgian mares are those given by Von Nathusius (6). The inheritance is what is usually called blending, but it was shown by Detlefsen (1) and others that such back crosses with apparently blending inheritance are susceptible of a Mendelian interpretation, on the basis of multiple factors incompletely dominant. If this interpretation of Landman's data (4) is correct, then heavy-horse types may contain factors for lighter bone. The heavy but heterozygous Gudbrandsdal horses may well give light cannon bone, for their history shows the infusion of much light-horse blood. The writer hesitates to advance the tentative hypothesis that segregation of size factors in horses may be influenced by age, but some such hypothesis is necessary to account for the poor performance of older stallions when compared with young. Race-horse stallions have been known to give excellent results when very old, for they are not bred for the dominant heavy-bone characters. Draft horses of the Gudbrandsdal and other heavy-horse breeds have not given similar satisfactory results. The reason may lie in the possibility that older stallions and mares are responsible for more recessives, whereas the younger give more dominants. Any such hypothesis, however suggestive, must be recast in the light of future investigations.

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LASPEYRESIA MOLESTA, AN IMPORTANT NEW INSECT ENEMY OF THE PEACH

[PRELIMINARY PAPER]

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INTRODUCTION

Attention is called to the discovery in the District of Columbia and environs of an important insect enemy of the peach believed to be new to the United States and apparently not heretofore known to science. Observations on this species by the writers during the summer and fall of 1916 warrant the fear that another formidable insect enemy of the peach and other deciduous fruits has become established in America. The insect is a moth belonging to the tortricid genus Laspeyresia, which contains numerous species of prime importance as pests in different parts of the world. Thus, *Laspeyresia funebrana* Tr. is the common plum worm or plum maggot of Europe and is said to be plentiful in plum pies. *L. woeberiana* Schiff. in Europe bores the bark of peach, cherry, plum, and apple trees. *L. nebritana* is the common pea moth, and *L. schistaceana* Sn. is a sugar-cane pest of importance in Java. In America the most important species is *L. pomonella* L., as yet better known under the generic name "Carpocapsa." The lesser apple worm, *L. prunivora* Walsh; the pecan moth, *L. caryana* Fitch; and *L. pyricolana* Murtfeldt are other familiar examples of the genus.

DESCRIPTION OF THE MOTH

Mr. August Busck, of the Bureau of Entomology, has prepared the following description of the species with comment on its relationships and possible origin:

Laspeyresia molesta, n. sp.

Head dark, smoky fuscous; face a shade darker, nearly black; labial palpi a shade lighter fuscous; antennæ simple, rather stout, half as long as the forewings, dark fuscous with thin, indistinct, whitish annulations. Thorax blackish fuscous; patagia faintly irrorated with white, each scale being slightly white-tipped. Forewings normal in form; termen with slight sinuation below apex; dark fuscous, obscurely irrorated by white-tipped scales; costal edge blackish, strigulated with obscure, geminate, white dashes, four very faint pairs on basal half and three more distinct on outer half besides two single white dashes before apex; from the black costal intervals run very obscure, wavy, dark lines across the wing, all with a strong outwardly directed wave on the middle of the wing; on the middle of the dorsal edge the spaces between three of these lines are more strongly irrorated with white than is the rest of the wing, so as to constitute two faint and poorly defined, white dorsal streaks. All these markings are only discernible in perfect specimens and under a lens; ocellus strongly irrorated with white, edged by two broad, perpendicular, faint bluish metallic lines and containing several small,

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deep black, irregular dashes, of which the fourth from tornus is the longest and placed farther outward, so as to break the outer metallic edge of ocellus; the line of black dashes as well as the adjoining bluish metallic lines are continued faintly above the ocellus in a curve to the last geminate costal spots; there is an indistinct, black apical spot and two or three small black dots below it; a thin but distinct, deep black, terminal line before the cilia; cilia dark bronzy fuscous. Hind wings dark brown with costal edge broadly white; cilia whitish; underside of wings lighter fuscous with strong iridescent sheen; abdomen dark fuscous with silvery white underside; legs dark fuscous with inner sides silvery; tarsi blackish with narrow, yellowish white annulations.

Alar expanse: 10 to 15 mm.

United States National Museum type 20664.

The present species is very similar to the European *Laspeyresia funebrana*, which is an important enemy of stone fruits in Europe, and it was at first supposed that it might be this European species which had been accidentally introduced into America, but several minor discrepancies both in the ornamentation of the moth and in the biology of the larva made this determination uncertain, and specimens were therefore submitted to the European specialists, Messrs. Edward Meyrick and J. H. Durrant, both of whom pronounced the species distinct from *L. funebrana* and unknown to them.

There are several American species closely allied to *Laspeyresia molesta*, but it is unlikely that the species is a native of this country; it has more probably been accidentally introduced from Japan, where closely allied species also occur, though the present species has not hitherto been reported. The theory of the Japanese origin is strengthened by a single specimen of a species of Laspeyresia which was reared from a shipment of pears from Japan to Seattle, Wash. The writers are unable to differentiate this specimen from those reared from peach in the East, and believe it to be the identical species.

Among the American species *Laspeyresia molesta* may easily be confused with (*Epinotia*) *Laspeyresia pyricolana* Murtfeldt, which not only is very similar both in adult and larval stages but which has similar biological habits and has also been reared from peach in the vicinity of Washington, D. C.

Laspeyresia molesta is, however, a larger and less mottled species, without the dark-brown transverse facia on the forewing found in *L. pyricolana;* the hind wings are more rounded, especially in the males, and not so triangular as in *L. pyricolana*. The males of *L. pyricolana* can at once be distinguished by a large patch of black scales on the upper surface near the base of the hindwings and by a similar black patch on the underside of the forewings; no such ornamentation is found in the males of *L. molesta*.

FULL-GROWN LARVA

Thirteen to fifteen mm. long; whitish suffused with pink; tubercles minute, black. Head light brown with darker brown markings; hind margin, ocellar area, and the tips of the trophi black. Thoracic shield light yellow, edged with brown. Spiracles small, circular, dark brown. Anal plate blackish fuscous. Legs and prolegs normal.

DISTRIBUTION OF THE SPECIES

So far as known to the writers, the insect in the United States is still confined to the general region of the District of Columbia. It is very generally present on peach trees in yards and elsewhere in the city of Washington and adjacent towns in Virginia and Maryland within a radius of 15 or 18 miles. Examples of injury to the peach by what is believed to be this insect have, however, been seen in the environs of Baltimore. The insect is thought to have been present in the District of Columbia for four or five years, or perhaps somewhat longer. Specimens of injured twigs were received at the Bureau of Entomology in the fall of 1913, and the work attributed to an unknown lepidopterous larva, although they are now believed to have been injured by *Laspeyresia molesta*. A few examples of injured twigs were received or collected during 1914 and 1915, but it was not until the fall of 1915 that its injuries were at all common. The writers were, unfortunately, not successful in obtaining adults from the larvæ until the spring of 1916, and the single specimen then obtained did not prove sufficient for identification purposes. During the summer of 1916, however, an abundance of adults were reared and certain observations made concerning the biology and injuries of the insect.

CHARACTER OF INJURY AND HABITS

The larvæ have been found injuring twigs of the peach (*Amygdalus persica*), plum (*Prunus* spp.), and cherry (*Prunus* spp.) and the fruit of the peach. The scarcity of the plum and cherry during 1916 in the infested area prevented observations as to the extent to which these fruits are attacked. The plum and cherry, however, have not shown such general infestation as observed for the peach, and it would appear that this latter is the insect's preferred food plant. It should be stated, however, that flowering cherries growing here and there in parks in Washington, especially the extensive plantings of Japanese flowering cherries in Potomac Park, are very generally infested. The twig injury to the cherry and plum is essentially the same as for the peach, though it is less conspicuous, due to less gum exudation (Pl. 26, A, B).

TWIG INJURY

In one peach orchard under observation an examination in mid-September showed that from 80 to 90 per cent of the twigs had been injured, and an even higher percentage of twigs of adjacent peach nursery stock had been attacked. Its injuries to the twigs of bearing orchards, while important as interfering with normal growth, are of less significance than the injuries of the caterpillars to the fruit. Twig injury in nurseries, however, is of much more importance, as the destruction of the terminal growing shoots results in the pushing out of shoots from lateral buds, producing a much-branched and bushy plant unsuitable for nursery stock (Pl. 27). Twig injury to newly planted orchards and to replants

in bearing orchards is also quite important, and aside from the actual injury inflicted would interfere a good deal with the proper shaping of the tree.

Attack on the twigs begins in the spring when the shoots are from 6 to 8 inches long and continues until active growth of the trees ceases in the fall. Many twigs injured in the latter part of the season present the appearance shown in Plate 28, B. As the twig hardens, the larva may leave its burrow and feed more or less on the exterior of the twig, cutting holes and pits into the bark and causing a copious exudation of gum, rendering the injury quite conspicuous. The more typical injury to twigs in the fall, however, is that represented in Plate 28, A.

The larvæ prefer tender, actively growing shoots, and their injury to these (Pl. 29, A, B) is scarcely distinguishable from that of the common peach-twig borer, or peach moth (*Anarsia lineatella* Zell). The caterpillars pass from one shoot to another in their search for appropriate food, and several shoots may thus be injured by a larva in the course of its growth. A striking illustration of this preference for tender growth was noted in an orchard near by in Virginia. Here the orchard trees had practically ceased growth, and although a large percentage of the twigs showed injury a careful search of these resulted in finding no larvæ. In an adjoining block of seedling nursery trees still growing vigorously larvæ in all stages were found very abundant. Injury to the shoots is apparently continuous during the active period of growth of the trees, even in the presence of fruit. The writers' observations are not conclusive as to whether the fruit is preferred to the twigs.

INJURY TO FRUITS

The fruit may be attacked while quite green, the infestation increasing as it approaches maturity. Larvæ of all sizes have been found abundantly in peaches during the ripening stage from midsummer on. Mid-season and early fall varieties have been noted as being worse infested, owing probably to the concentration of larvæ on the fruit by reason of the cessation of active growth of the twigs. Thus, in the case of some Salway and Smock trees and certain varieties of clingstone peaches, of similar season, practically all the fruit on the trees was infested with from one to three or four larvæ.

In attacking the fruit the young caterpillars rather generally eat through the skin at or near the point of attachment of the fruit stem, the place being indicated by more or less frass adhering to the surface of the fruit (Pl. 30, B). Entrance is also made at other places, especially where the fruit has been punctured by the curculio or abrased by limbs or branches or other causes, as by hail. If the fruit is ripe, or nearly so, the entrance point of the larva may soon be invaded by the brownrot fungus, the larva continuing its development, in frequent instances, in the fungus-invaded and decaying flesh of the peach. Owing to the com-

bined effect of the caterpillar and brownrot fungus, a good deal of fruit may fall to the ground, though the majority of the fruit infested by the caterpillars will remain hanging on the trees, especially if the fruit was invaded when nearly mature. If the peach be entered at the stem end, the larva as it grows makes its way to the pit, where it feeds on the flesh, which soon becomes much discolored and more or less slimy (Pl. 31). Larvæ entering at the side of the fruit are more likely to eat out pockets or cavities in the flesh, as shown in Plate 30, A. The inconspicuous entrance holes of the young larvæ, especially at the stem end, often render it difficult to detect wormy fruit by exterior examination. In numerous cases apparently sound fruit when cut open has been found infested with one or more larvæ.

PUPATION AND HIBERNATION

The caterpillar when full grown seeks some protected place where a cocoon of whitish silk is made preparatory to pupation. Cocoons in summer have been found in the cavity at the stem end of the fruit (Pl. 30, B), between fruits in contact, on or between mummified peaches, in leaves gummed to the twigs, or similar situations. It is probable that many larvæ find protected places on the twigs, in cracks, under bark scales on the trunk and branches, and in débris on the soil. During September larvæ were frequently observed making their way into the cracks in the bark of the trunk and larger limbs of the peach, evidently seeking winter quarters. Winter cocoons have been found in a few instances in little cavities eaten into the bark at the tips of injured twigs and more or less protected by the dried exuded gum and attached leaf fragments. The larvæ in general appear to be rather indiscriminate in their choice of pupation quarters and may be expected to choose any place on the trees where protection is afforded. Many larvæ have been collected under bands of burlap wrapped around the trunk and larger limbs of the trees. In the case of nursery stock the absence of rough bark and other protection on the young trees probably forces the larvæ to the ground, though a few individuals might find protection here and there on the plants. The insect hibernates in the full-grown larval condition in silken cocoons, pupation occurring in the spring. Owing to its manner of hibernating, the detection of the insect on nursery stock and young trees would be extremely difficult, and the disinfection of trees from the pest could be insured only by adequate fumigation with hydrocyanic-acid gas or other suitable substance.

EMERGENCE OF MOTHS AND NUMBER OF GENERATIONS.

Moths are out egg laying in the spring by the time the shoots of the peach are well out, as the work of the larvæ is in evidence when the shoots are 6 or 8 inches long. It would appear that there are two and probably three broods of larvæ each year, since injury begins early in the season, and larvæ in various stages of growth are to be found in late fall.

PLATE 26

Laspeyresia molesta:

A.—Injury to shoot of a Domestica plum.
B.—Injury by larva to cherry.

A
B

PLATE 27

Laspeyresia molesta:

One-year budded peach nursery tree, showing injury of caterpillars.

PLATE 28

Laspeyresia molesta:

A.—Typical appearance of peach twigs in fall injured by larva.

B.—Peach twig, showing large mass of dried gum and leaf fragments due to attack by the caterpillar.

A
B

A
B

PLATE 29

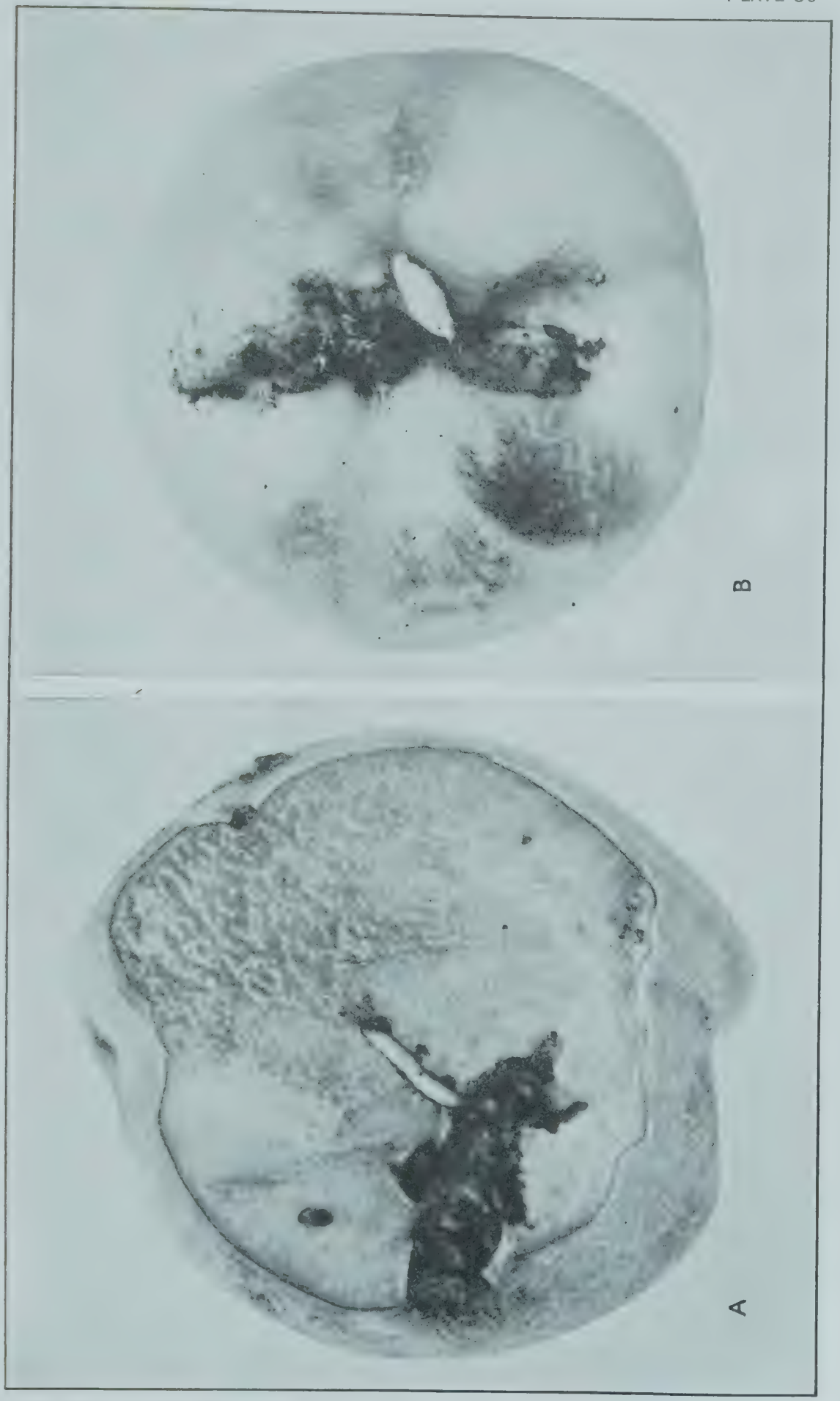
Laspeyresia molesta:

A.—Typical exterior appearance of larval injury to peach shoot.
B.—The same shoot cut open, showing the larva in its burrow.

PLATE 30

Laspeyresia molesta:

A.—Cavity excavated in peach by larva entering at the side.
B.—Larval injury at stem end of peach; also the summer cocoon of the insect.


B
A

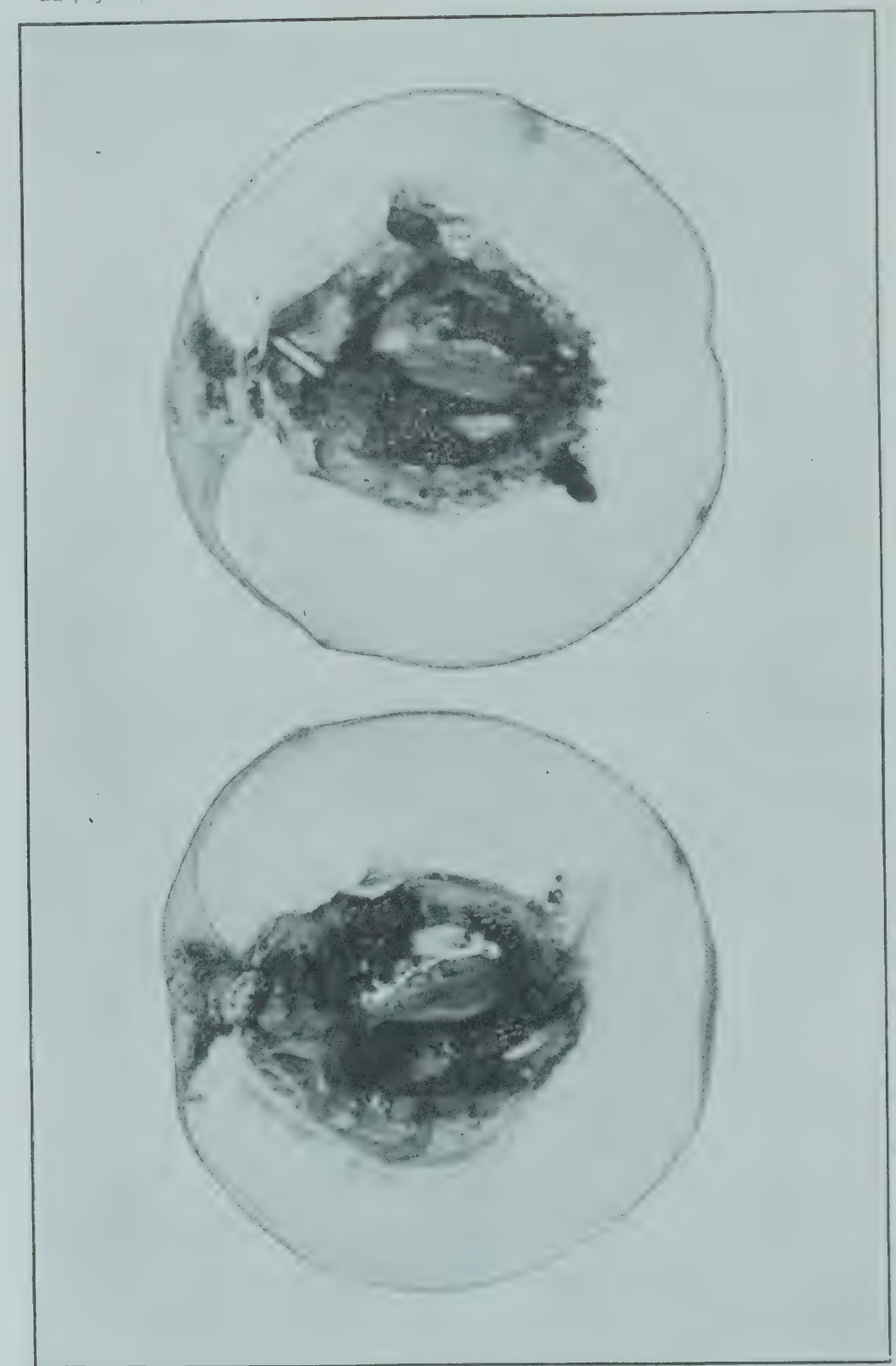


PLATE 31

Laspeyresia molesta:

Peach cut open to show larval injury at the pit.

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ENERGY VALUES OF RED-CLOVER HAY AND MAIZE MEAL

By HENRY PRENTISS ARMSBY, *Director*, J. AUGUST FRIES, *Assistant Director*, and WINFRED WAITE BRAMAN, *Associate, Institute of Animal Nutrition of The Pennsylvania State College*

COOPERATIVE INVESTIGATIONS BETWEEN THE BUREAU OF ANIMAL INDUSTRY OF THE UNITED STATES DEPARTMENT OF AGRICULTURE AND THE INSTITUTE OF ANIMAL NUTRITION OF THE PENNSYLVANIA STATE COLLEGE

INTRODUCTION

The results of determinations at this institute of the net energy values for cattle of 10 different feeding stuffs or mixtures were reported by Armsby and Fries in a previous paper.[1] Attention was there called to the discordant results obtained for red-clover hay and for maize meal, and certain of them were tentatively rejected, for reasons stated, in making up the final averages. The present experiments were undertaken in order to obtain additional data concerning the energy values of these feeding stuffs. They were conducted in 1915 along the lines of the previous experiments just referred to.

GENERAL DESCRIPTION OF EXPERIMENTS

The general plan of the experiments was to feed five different rations. The first two consisted of two different amounts of red-clover hay alone, one a submaintenance ration, and the other a heavy ration. The remaining rations contained different amounts of a mixture of one-third clover hay and two-thirds maize meal, one amount being much below maintenance, one approximately maintenance, and one a heavy ration. Ten gm. of salt were added to each day's ration.

The animal used was a pure-bred Shorthorn steer, 2 years old, weighing at the beginning of the experiments a little over 500 kgm.

As in previous experiments, each feeding period covered 21 days, the first 11 being preliminary and the last 10 the digestion period proper. Table I gives the dates and the rations for the several periods and also the live weights of the animals.

[1] Armsby, H. P., and Fries, J. A. Net energy values of feeding stuffs for cattle. *In* Jour. Agr. Research, v. 3, no. 6, p. 435–491. 1915.

TABLE I.—*Duration of experiments in 1915, rations fed, and live weight of the animals*

Period No.	Preliminary period.	Digestion period.	Ration.		Live weight of steer.
			Clover hay.	Maize meal.	
			Gm.	*Gm.*	*Kgm.*
I	Jan. 3–13	Jan. 14–23	7,000	.....	513.5
II	Jan. 24–Feb. 3	Feb. 4–13	4,500	.....	497.2
III	Feb. 21–Mar. 3	Mar. 4–13	1,500	3,000	489.5
IV	Mar. 21–31	Apr. 1–10	2,500	5,000	513.5
V	Apr. 11–21	Apr. 22–May 1	1,000	2,000	491.2

The hay used was grown on the college farm and cut when in full bloom. It graded as "good hay." It was fed cut in lengths of 5 to 10 cm. The maize meal was ground from No. 2 yellow corn.

EXPERIMENTAL METHODS

The experimental and analytical methods in these experiments were the same as those previously given in detail,[1] with the exception of the determinations of carbon and hydrogen. The total carbon in the urines was determined directly by combustion of the liquid urine by a method worked out in this laboratory[2] and that of the feed and feces by combustion in a bomb calorimeter as described by Fries.[3] The organic hydrogen was not determined, as it has been found that the error resulting from omitting it entirely from the computation is very small.

Table II shows the average composition of the dry matter of the feeding stuffs used.

TABLE II.—*Composition of the dry matter of the feeding stuffs*

Feeding stuff and period No.	Ash.	Protein.	Nonprotein.	Crude fiber.	Nitrogen-free extract.	Ether extract.	Heat of combustion per kilogram.
Clover hay:	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*Calories.*
I and II	5.81	8.26	1.11	34.34	48.54	1.95	4,367
III	6.20	9.49	1.25	31.40	49.33	2.34	4,403
IV and V	5.79	9.94	1.03	30.56	50.14	2.55	4,407
Average	5.93	9.23	1.13	32.10	49.34	2.28	4,393
Maize meal:							
III	1.44	9.74	.35	2.04	82.13	4.30	4,515
IV and V	1.37	9.82	.20	2.04	82.21	4.36	4,496
Average	1.41	9.78	.28	2.04	82.17	4.33	4,505

[1] Armsby, H. P., and Fries, J. A. The influence of type and of age upon the utilization of feed by cattle. U. S. Dept. Agr. Bur. Anim. Indus. Bul. 128, p. 203. 1911.

[2] Braman, W. W. A study in drying urine for chemical analysis. *In* Jour. Biol. Chem., v. 19, no. 1, p. 105–113. 1914.

[3] Fries, J. A. The determination of carbon by means of the bomb calorimeter. *In* Jour. Amer. Chem. Soc., v. 31, no. 2, p. 272–278, 1 fig. 1909.

PERCENTAGE DIGESTIBILITY OF RATIONS

From the daily records of feed and excreta and their chemical composition the percentage digestibility of the several rations has been computed in the usual manner, with the results shown in Table III.

It was assumed that in the mixed rations the hay had the percentage digestibility shown by the average of the periods when hay was fed alone, and the percentage digestibility of the maize meal in Periods III, IV, and V has also been computed, with the results shown in the last three columns of the table.

TABLE III.—*Digestibility of the rations*

Constituent.	Percentage digestibility of rations.								
	Clover hay.		Clover hay and maize meal.			Average percentage digestibility of clover hay.	Computed percentage digestibility of maize meal.		
	Period I.	Period II.	Period III.	Period IV.	Period V.	Periods I and II.	Period III.	Period IV.	Period V.
Dry matter	55.88	59.26	78.78	73.22	79.20	57.23	89.78	81.54	90.63
Ash	28.57	31.62	41.94	27.22	27.94	29.78	68.72	21.54	23.75
Organic matter	57.56	60.97	79.94	74.58	80.72	58.92	90.15	82.37	91.55
Protein	35.29	41.24	62.44	55.86	63.00	37.66	74.74	65.46	76.34
Crude fiber	50.23	53.19	54.22	47.54	46.55	51.41	76.27	'17.42	8.68
Nitrogen-free extract	66.24	69.76	87.19	82.04	89.13	67.64	93.19	86.61	95.95
Ether extract	49.14	54.29	78.10	76.30	81.49	51.19	85.52	83.96	90.68
Total nitrogen	42.87	46.33	60.82	53.85	62.01	44.25	69.68	59.21	71.77
Carbon	54.10	57.50	77.85	72.41	78.32	55.46	89.32	81.25	90.23
Energy	53.80	57.20	77.28	71.87	77.65	55.15	88.29	80.40	89.12

METABOLIZABLE ENERGY

The difference between the chemical energy of the feed and that lost in the excreta shows how much of the former is capable of transformation in the animal body. This has been called metabolizable energy.

Computed in the same manner as in the earlier paper, the losses of chemical energy per kilogram of dry matter consumed and the metabolizable energy remaining were as shown in Table IV, which includes also the percentage distribution of the feed energy between the various excreta on the one hand and the metabolizable energy on the other.

The average results for the metabolizable energy per kilogram of dry matter and per kilogram of digestible organic matter are brought together for convenience in Table V.

TABLE IV.—*Losses of energy and their percentage distribution*

Feed and period No.	Dry matter eaten per head and per day.		Energy per kilogram of dry matter.				Metabolizable energy.	Metabolizable energy per kilogram of digestible organic matter.	Percentage losses.			Percentage metabolizable.
			Total.	Losses.								
	Coarse feed.	Concentrate.		In feces.	In urine.	In methane.			In feces.	In urine.	In methane.	
Clover hay:	*Gm.*	*Gm.*	*Cal.*	*Cal.*	*Cal.*	*Cal.*	*Cal.*	*Cal.*				
I	5,952	0	4,367	2,018	153	287	1,909	3,522	46.20	3.51	6.57	43.72
II	3,942	0	4,367	1,869	172	304	2,022	3,522	42.80	3.93	6.96	46.31
True average	.....	.....	4,367	1,958	161	294	1,954	3,522	44.85	3.68	6.72	44.75
Clover hay and maize meal:												
III	1,328	2,602	4,477	1,017	173	413	2,874	3,708	22.72	3.86	9.23	64.19
IV	2,272	4,363	4,465	1,256	136	340	2,733	3,733	28.13	3.05	7.61	61.21
V	909	1,748	4,465	998	192	495	2,780	3,546	22.35	4.30	11.09	62.26
True average	.....	.....	4,469	1,133	159	393	2,764	3,705	25.26	3.55	8.79	62.30
Maize meal computed:												
III	1,328	2,602	4,515	529	179	473	3,334	3,753	11.71	3.96	10.48	73.85
IV	2,272	4,363	4,496	881	133	363	3,129	3,851	19.60	3.73	8.07	69.60
V	909	1,748	4,496	489	208	599	3,200	3,543	10.88	4.63	13.32	71.17
True average	.....	.....	4,501	697	157	443	3,204	3,755	15.49	3.40	9.85	71.18

TABLE V.—*Average losses of chemical energy and metabolizable energy*

Feed and period No.	Gross energy per kilogram of dry matter.	Losses of chemical energy per kilogram of dry matter.	Metabolizable energy.	
			Per kilogram of dry matter.	Per kilogram of digestible organic matter.
Clover hay:	*Calories.*	*Calories.*	*Calories.*	*Calories.*
I	4,367	2,458	1,909	3,522
II	4,367	2,345	2,022	3,522
True average	4,367	2,413	1,954	3,522
Maize meal computed:				
III	4,515	1,181	3,334	3,753
IV	4,496	1,367	3,129	3,851
V	4,496	1,296	3,200	3,543
True average	4,501	1,297	3,204	3,755
Clover hay and maize meal:				
III	4,477	1,603	2,874	3,708
IV	4,465	1,732	2,733	3,773
V	4,465	1,685	2,780	3,546
True average	4,469	1,685	2,784	3,705

A comparison of the metabolizable energy per kilogram of digested organic matter as given in Table IV with the previous results shows a very close agreement, the figures being for clover hay 3.52 therms as compared with 3.49 therms, and that for maize meal 3.76 therms as compared with 3.80 therms.

INFLUENCE OF QUANTITY OF FEED CONSUMED ON LOSSES OF CHEMICAL ENERGY

A study of the percentage losses of chemical energy substantially confirms the earlier results regarding the influence of the quantity of feed upon these losses. If Periods I and II, in which a large and a much smaller hay ration were fed under similar conditions, are compared, it is seen that with the smaller ration the losses in the urine and in the methane were decidedly greater and the loss in the feces less than with the larger ration.

Periods III, IV, and V are similarly comparable, the rations of hay and maize meal being fed under the same conditions but in varying quantities. Here, as in the periods when hay alone was fed, the lightest ration shows the greatest loss in urine and methane and the least in the feces, while with the heaviest ration the reverse was true. The losses computed for the maize meal alone show differences in the same direction.

As regards variation in the percentage of total energy which was metabolizable there were slight differences. In the case of both the hay rations and the mixed rations the largest feed gave the smallest percentage, but with the mixed ration the smallest feed did not give the largest percentage. As in the experiments previously reported, the quantity of feed failed to show any definite effect upon the percentage of energy metabolized.

METHANE PRODUCTION

The relation of the methane to the digestible carbohydrates has been found to be fairly constant, so that an average figure may be used to estimate the combustible gases in the absence of the costly apparatus necessary for their actual determination.

Table VI gives this relation as actually found in these experiments.

TABLE VI.—*Quantity of methane per 100 gm. of digestible carbohydrates*

Feed and period No.	Carbohydrates.	Methane.	Methane per 100 gm. of digestible carbohydrates.	Average.
Clover hay:	*Gm.*	*Gm.*	*Gm.*	*Gm.*
I	2,940.6	127.9	4.35	4.36
II	2,054.4	89.8	4.37	
Hay and maize meal:				
III	2,689.2	121.7	4.53	4.63
IV	4,249.3	169.1	3.98	
V	1,832.4	98.6	5.38	

If these figures are compared with those of the previous experiments it is seen that while Periods I and II agree very closely, the average is somewhat lower than the earlier average for clover hay, 4.6 gm. The results from Periods III, IV, and V vary rather widely, although their average, 4.63 gm., is only a little lower than the average of the previous experiments, 4.8 gm.

HEAT PRODUCTION

The daily heat production measured and that computed in the usual manner from the balance of carbon and nitrogen are compared in Table VII.

TABLE VII.—*Observed and computed daily heat production*

Period.	Observed.	Computed.	Error.	Computed ÷ observed.
	Calories.	*Calories.*	*Calories.*	*Per cent.*
I, first day	12,238.7	12,128.7	−110.0	99.10
I, second day	12,008.4	11,634.3	−374.1	96.88
II, first day	10,389.1	10,068.9	−320.2	96.92
II, second day	10,187.1	10,148.5	− 38.6	99.62
III, first day	10,761.9	10,848.7	+ 86.8	100.81
III, second day	10,746.5	10,770.6	+ 24.1	100.22
IV, first day	13,757.7	13,574.3	−183.4	98.67
IV, second day	13,930.3	13,474.3	−456.0	96.73
V, first day	9,910.2	10,076.8	+166.6	101.68
V, second day	10,201.4	10,195.8	− 5.6	99.95

ANALYSIS OF HEAT PRODUCTION

Standing and lying have been found to exert such an influence on the heat production of animals that in order to make comparisons the observed results must be corrected to a uniform proportion of time standing and lying. The total heat production for each day of the 2-day periods has therefore been corrected to 12 hours' standing and 12 hours' lying in the manner described in the previous paper [1] and the two days averaged, and the distribution of this corrected heat production also has been computed by the method explained on page 468 of the publication just referred to.[1] The results of these computations are recorded in Table VIII.

TABLE VIII.—*Heat production per day per head corrected to 12 hours' standing*

Period No.	Dry matter eaten.		Total heat production (average of 24 hours).	Distribution of heat production.			
	Hay.	Grain.		Standing.	Rising and lying down.	Fermentation.	Remainder.
	Gm.	*Gm.*	*Cal.*	*Cal.*	*Cal.*	*Cal.*	*Cal.*
I	5,952.3	.....	12,251.3	1,584	72	777	9,818
II	3,941.5	.....	10,332.9	1,287	110	545	8,391
III	1,327.9	2,601.7	11,100.8	1,411	112	739	8,839
IV	2,271.7	4,363.0	14,129.0	1,879	127	1,026	11,097
V	908.7	1,747.5	9,854.7	1,183	113	598	7,961

[1] Armsby, H. P., and Fries, J. A. Net energy values of feeding stuffs for cattle. In Jour. Agr. Research v. 3, no. 6, p. 454. 1915.

ENERGY EXPENDITURE PER KILOGRAM OF DRY MATTER

A comparison of Periods I and II shows how much each additional kilogram of dry matter of the hay consumed increased the total heat production and its several factors (Table IX).

TABLE IX.—*Computation of energy expenditure per kilogram of clover hay*

Period No.	Quantity of dry matter eaten.	Total heat production	Distribution of heat production.			
			Standing.	Rising and lying down.	Fermentation.	Remainder.
	Gm.	*Cal.*	*Cal.*	*Cal.*	*Cal.*	*Cal.*
I	5,952.3	12,251.3	1,584	72	777	9,818
II	3,941.5	10,332.9	1,287	110	545	8,391
Difference	2,010.8	1,918.4	297	−38	232	1,427
Difference per kilogram of dry matter	.....	954.0	148	−19	115	710

In making the computation for the maize meal fed in Periods III, IV, and V, when hay and meal were fed, the increase in the heat production due to the differences in the quantity of hay consumed, computed by the use of the value per kilogram of dry matter just obtained, has to be subtracted from the total increment in the manner shown in the following example (Table X):

TABLE X.—*Computation of energy expenditure per kilogram of maize meal*

Period No.	Quantity of dry matter eaten.		Total heat production.	Distribution of heat production.			
	Hay.	Grain.		Standing.	Rising and lying down.	Fermentation.	Remainder.
	Gm.	*Gm.*	*Cal.*	*Cal.*	*Cal.*	*Cal.*	*Cal.*
Period IV	2,271.7	4,363.0	14,129.0	1,879	127	1,026	11,097
Period V	908.7	1,747.5	9,854.7	1,183	113	598	7,961
Difference	1,363.0	2,615.5	4,274.3	696	14	428	3,136
Difference due to 1,363.0 gm. of hay	.....	.....	1,300.3	202	−26	157	968
Difference due to 2,615.5 gm. of grain	.....	.....	2,974.0	494	+40	271	2,168
Difference per kilogram of grain	.....	.....	1,137.1	189	15	104	829

Six comparisons according to this method are possible, affording the results given in Table XI.

TABLE XI.—*Increment of heat production per kilogram of dry matter*

Feeding and period No.	Total increment.	Analysis of heat increment.			
		Standing 12 hours.	Rising and lying down.	Methane fermentation.	Remainder.
Clover hay:	*Cal.*	*Cal.*	*Cal.*	*Cal.*	*Cal.*
I–II	954	148	−19	115	710
Maize meal:					
III–II	1,253	196	−18	190	885
IV–II	1,235	192	− 3	154	892
V–II	1,382	197	−31	230	986
IV–III	1,208	186	+19	101	902
III–V	990	194	+ 8	109	679
IV–V	1,137	189	+15	104	829
Average of all	1,201	192	− 2	148	862
Average, omitting Periods III–II and II–V	1,143	190	10	117	826

The total energy expenditure per kilogram of dry matter of the clover hay, 954 Calories, agrees well with the value 992 Calories previously obtained for clover hay in experiment 179, and indicates that the very low value of 453 Calories obtained in experiment 186 was, as was suspected, erroneous.[1] It would appear that the mean of the two, 973 Calories, may be taken as the average value for red-clover hay, particularly as it is only slightly higher than that of 932 Calories computed from one of Kellner's experiments.[2]

The average figure for the total increment per kilogram of dry matter of maize meal eaten is 1,201 Calories, somewhat lower than the value of 1,434 Calories previously published. Another earlier experiment gave a value of 952 calories; but this was discarded, since the increment during lying (the "remainder" of Table XI) was only 393 Calories, as compared with 906 Calories in the experiment reported and 863 Calories, the average obtained in the present series.

Of the comparisons tabulated, however, Periods III–II and Periods II–V, are based on comparatively small differences in total heat production, so that the deduction for the energy expenditure due to the hay enters as a relatively large factor. If these two comparisons are omitted, the average of the remaining four is 1,143 Calories, which agrees closely with that of 1,137 Calories obtained from a comparison of the lightest and heaviest mixed rations (Periods IV–V), so that we are inclined to attach greater weight to this figure. The mean of this and the earlier experiment is 1,289 Calories, which may be taken as the corrected value for the heat production caused by the consumption of 1 kgm. of dry matter of maize meal by cattle.

[1] Armsby, H. P., and Fries, J. A. Op. cit., 1915, p. 473, 482.
[2] Idem., p. 478.

NET ENERGY VALUES

In computing, finally, the net energy values, by subtracting the sum of the losses of chemical energy and the energy expended in feed consumption from the gross energy of the feed, we have used first the values for the energy expended in feed consumption obtained in these experiments and then have made a second computation, using instead the average between these values and those obtained in previous experiments as computed in the foregoing paragraph. Table XII gives the final results.

TABLE XII.—*Net energy values of feeding stuffs per kilogram of dry matter*

Feed.	Gross energy.	Losses of chemical energy.	Energy expended in feed consumption.	Net energy values.
Clover hay:	*Cal.*	*Cal.*	*Cal.*	*Cal.*
These experiments	4,367	2,413	954	1,000
Mean	4,367	2,413	973	981
Maize meal:				
These experiments	4,501	1,297	1,143	2,061
Mean	4,501	1,297	1,289	1,913

SUMMARY

Results are here reported of five feeding periods with cattle, two with differing amounts of clover hay alone and three with clover hay and maize meal in differing quantities.

(1) The metabolizable energy per kilogram of digested organic matter was found to be 3.52 therms for the clover hay and 3.76 therms for the meize meal as compared with 3.49 therms and 3.80 therms, respectively, as previously reported.

(2) The average increment in heat production caused by the consumption of 1 kgm. of dry matter was as follows:

	Calories
(*a*) For clover hay	954
(*b*) For maize meal	1,143

(3) When these results are combined with those of previous experiments, the following corrected values for the average heat increment per kilogram dry matter are computed:

	Calories
(*a*) For clover hay	973
(*b*) For maize meal	1,289

(4) The average net energy values per kilogram of dry matter obtained by the use of the foregoing averages were:

	Calories
(*a*) For clover hay	981
(*b*) For maize meal	1,91

RELATIONSHIP BETWEEN THE WETTING POWER AND EFFICIENCY OF NICOTINE-SULPHATE AND FISH-OIL-SOAP SPRAYS

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INTRODUCTION

The influence of the wetting power upon the efficiency of a contact insecticide has never been entirely determined, although it has long been realized that this quality is an important limiting factor in the efficacy of certain sprays in killing insects, especially aphids, whose bodies are more or less covered with a waxy secretion. The difficulty of determining the wetting power of a solution has in the past precluded its consideration in the comparison of contact sprays in the laboratory. The present work upon the relationship of wetting power to the efficiency of nicotine and soap solutions developed from experiments performed during 1914 and 1915 in spraying garden peas (*Pisum sativum*) for the control of the green-pea aphid [(*Macrosiphum*) *Acyrthosiphum pisi* Kalt.], spinach (*Spinacia oleraceae*) for the control of the spinach aphid (*Myzus persicae* Sulz.), and strawberries (*Fragaria* sp.) for the control of red spiders (*Tetranychus* sp.). The results of the experiments demonstrated that the optimum efficiency of sprays containing nicotine sulphate and fish-oil soap was reached with a definite degree of concentration [2] and that solutions which were more concentrated and also those of lower concentration were less effective in killing the insects. In order to avoid complicated conditions, the following charts of the efficiency of the sprays are based altogether on the results of the pea-spraying experiments. The proportional efficiency of the sprays against the spinach aphids and red spiders was almost identical with the results obtained on the pea aphids.

When nicotine sulphate and fish-oil soap are mixed before they are diluted, under ordinary conditions of temperature, a precipitate may be formed. This fact has previously been noted by other authors.[3] The composition of the precipitate is probably unknown, although it is generally supposed that the resulting solutions are less effective killing agents than when the materials are mixed in dilute solutions. From observa-

[1] Detailed by the Virginia Crop Pest Commission for the investigation of insects affecting truck crops.

[2] The terms "concentration" and "concentrated solutions" as used in this paper refer to the amounts of materials called for by the various formulæ, and not to the original insecticides previous to their dilution, unless so stated.

[3] Parker, W. B. The hop aphis in the Pacific region. U. S. Dept. Agr. Bur. Ent. Bul. 111, p. 27. 1913.

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tions in the field it was noticed that certain sprays of high concentrations of soap apparently did not wet the insects as thoroughly or spread over the glaucous leaves of the pea vines as well as less concentrated soap and nicotine solutions. It was also noted that the solutions of high concentrations were not as effective insecticides as were some of the more dilute mixtures.

METHODS OF DETERMINING THE EFFICIENCY AND WETTING POWERS OF THE SOLUTIONS

The efficiency, or the killing power, of the solutions was determined from actual field-spraying experiments. Peas were sprayed five times, spinach once, and strawberries once. The experiments were performed on 27 one-twentieth-acre plots. A power sprayer which maintained a pressure of 75 to 125 pounds was used to apply the materials. Three nozzles per row were employed, two lateral and one vertical. Before the spray was applied, the number of live aphids on the vines were counted for a certain distance in the center of the plot. Two hours after spraying, another determination of the number of live insects was made on the same vines as before, and from these figures the percentage of the insects killed was computed. When spraying strawberries to control the red spider, the number of live and dead mites on several leaves from each plant were counted, and the efficiency determined in this way. Detailed results of this work are shown in Table I.

The comparative wetting powers of the spray solutions were determined by the method recommended by Cooper and Nuttall.[1] In this method a standard paraffin oil having a density of 0.8690 is run from a pipette through the solution to be tested, and the number of drops formed from a definite volume of oil are counted. The wetting power is directly proportional to the drop number. It is advisable to use distilled water as a standard liquid, as there is variation in different samples of oil; hence, the wetting power of the solutions is expressed as the ratio of the drop number of the solution to that of distilled water multiplied by 100.

The determination of the percentage of nicotine in the solutions was made by a test which was approved and adopted by the Bureau of Animal Industry on March 1, 1915. The method is well known and needs no description here.

[1] Cooper, W. F., and Nuttall, W. H. The theory of wetting, and the determination of the wetting power of dipping and spraying fluids containing a soap basis. *In* Jour. Agr. Sci., v. 7, pt. 2, p. 235. 1915.

TABLE I.—*Combined data of spraying experiments with nicotine sulphate and fish-oil soap in 1914 and 1915 at Norfolk, Va.*

Group No.	Plot.	(*Macrosiphum*) *Acyrthosiphum pisi* (5 sprayings).			Number of *Myzus persicae* killed.	Number of *Tetranychus* sp. killed.	Wetting power of solutions.	Nicotine in the sprays.
		Number alive before spraying.	Number alive after spraying.	Number killed.				
				Per ct.	*Per ct.*	*Per ct.*		*Per ct.*
1	13	1,343	336	75.0	72.5	79.6	103	0.0650
1	9	1,614	308	80.9	82.0	83.3	193	.0650
1	4	1,567	144	90.8	88.9	90.8	615	.0650
1	10	1,830	242	86.8	85.2	86.9	628	.0650
1	11	1,353	199	85.3	85.9	86.9	743	.0650
1	12	1,359	270	80.1	79.8	82.8	750	.0650
2	6	1,289	320	75.2	75.1	80.0	788	.0215
2	7	1,191	355	70.2	66.5	76.8	754	.0260
2	3	1,282	83	93.5	93.8	95.0	743	.0425
2	8	1,246	51	95.9	95.1	95.0	732	.0575
2	10	1,830	242	86.8	85.2	86.9	628	.0650
3	18	1,462	934	36.1	34.9	60.5	181	.0215
3	17	1,531	741	51.6	52.1	66.4	175	.0260
3	16	1,391	522	62.5	59.9	71.4	163	.0325
3	15	1,435	445	69.0	69.7	76.9	154	.0425
3	14	1,406	404	71.3	70.3	76.9	125	.0575
3	13	1,343	336	75.0	72.5	79.6	103	.0650
4	19	1,499	955	36.3	34.7	60.6	307	0
4	20	1,579	846	46.4	45.3	68.8	363	0
4	21	1,681	891	47.0	44.9	69.0	450	0
4	22	1,406	600	57.3	55.2	64.4	645	0
4	23	1,564	575	63.2	60.1	74.5	689	0
4	24	1,344	497	63.0	60.3	71.5	1,067	0
4	25	1,487	391	73.7	75.0	78.0	1,080	0
4	27	1,334	331	75.2	74.2	82.0	1,106	0
4	27	1,466	365	75.1	74.8	82.0	1,112	0

FORMULÆ TESTED

In the preparation of contact sprays to be applied to tender plants the concentration of the solutions is limited between a minimum which is a strength sufficient to kill all the insects which it strikes and a maximum which is the greatest concentration that can be applied without injury to the foliage. In the experiments on peas or young spinach the greatest concentration which could be used without injuring the plants was 8 pounds of soap [1] to 50 gallons of water, or a 1-to-534 concentration of nicotine sulphate. The minimum concentration was not so sharply defined.

It was the writer's endeavor to try, so far as possible, such practical combinations of the two materials as were at all likely to give satisfactory results. In order to make the formulæ more comprehensive, they

[1] A standard caustic-potash fish-oil soap was used throughout these experiments.

are placed below in their logical groups, 50 gallons of water being used in each case.

GROUP 1.—A constant amount of nicotine sulphate, with which fish-oil soap was used in varying quantities.

Plot.	Nicotine sulphate.		Fish-oil soap.
	Ounces.	*Ratio.*	*Pounds.*
13	10	1:630	1
9	10	1:630	3
4	10	1:630	4
10	10	1:630	5
11	10	1:630	6
12	10	1:630	7

GROUP 2.—A constant amount of fish-oil soap, with which nicotine sulphate was used in varying quantities.

Plot.	Fish-oil soap.	Nicotine sulphate.	
	Pounds.	*Ounces.*	*Ratio.*
6	5	3¼	1:1,938
7	5	4	1:1,575
3	5	6½	1:969
8	5	8¾	1:720
10	5	10	1:630

GROUP 3.—A minimum constant amount of soap, to which nicotine sulphate was added in varying quantities.

Plot.	Fish-oil soap.	Nicotine sulphate.	
	Pounds.	*Ounces.*	*Ratio.*
18	1	3¼	1:1,938
17	1	4	1:1,575
16	1	5	1:1,260
15	1	6½	1:969
14	1	8¾	1:720
13	1	10	1:630

GROUP 4.—Fish-oil soap used alone.

Plot.	Fish-oil soap.	Plot.	Fish-oil soap.
	Pounds.		*Pounds.*
19	2	24	5
20	3	25	6
21	3½	26	7
22	4	27	8
23	4½		

EXPERIMENTAL WORK

GROUP 1

The effectiveness of the sprays containing nicotine sulphate in the ratio of 1 to 630 with various amounts of soap to 50 gallons of solution is represented by the efficiency curve for this group in figure 1. The curve begins with 1 pound of soap to 50 gallons of spray, at 75 per cent efficiency. It gradually rises with the increased amounts of soap in the formulæ to 90.8 per cent, which is the efficacy of 4 pounds of soap plus 50 gallons of a 1-to-630 solution of nicotine sulphate. The solutions containing greater concentrations of soap than the above lose effectiveness, and the curve drops to 85.3 per cent at 6 pounds and to 80.1 per cent for the formula which contained 7 pounds of soap.

The degree of wetting of the solutions in group 1 is shown by a curve in figure 1. This is based on the arbitrary comparative values in the column at the right. The wetting power of the formula containing 1 pound of soap to 50 gallons of 1-to-630 nicotine solution is 103, the curve then rises to 193 for the formula containing 3 pounds of soap. A sudden increase in the wetting power takes place at this point, the curve going to 615 for 4 pounds, 628 for 5 pounds, 743 for 6 pounds, and 750 for 7 pounds of soap to the 50 gallons of 1-to-630 nicotine-sulphate solution.

GROUP 4

Group 4 is one of eight formulæ for fish-oil-soap solutions at ratios between 2 and 8 pounds to 50 gallons of water. The efficiency curve for group 4 is given in figure 1. The efficacy of a solution of 2 pounds of fish-oil soap to 50 gallons of water is 36.3 per cent. From this the curve rises to 73.7 per cent for a solution which contained 6 pounds of soap to 50 gallons. Greater concentration of the solutions gave but slight increase in the effectiveness, as is shown by the curve, which remains only a fraction above 75 per cent for the solutions containing 7 and 8 pounds of soap.

The wetting-power curve of the solutions in group 4 is shown in figure 1. The curve begins at 307 for the solution containing 2 pounds of soap and rises gradually to 363 for 3 pounds of soap. From this point the wetting power being greatly increased by further additions of soap, the curve rises to 645 for 4 pounds and 1,067 for 5 pounds of soap. Further concentration of the solutions increased the wetting power very little, a solution of 8 pounds of soap having the wetting value of 1,112.

DISCUSSION OF GROUPS 1 AND 4

The results of these experiments indicate (1) that the addition of nicotine sulphate to fish-oil-soap solutions decidedly increases their effectiveness in destroying aphids; (2) that the efficiency of nicotine

sprays can be increased to a considerable degree by the addition of soap, but when more than 4 pounds of soap are used to 50 gallons of 1-to-630 nicotine-sulphate solution the effectiveness of these solutions decreases;

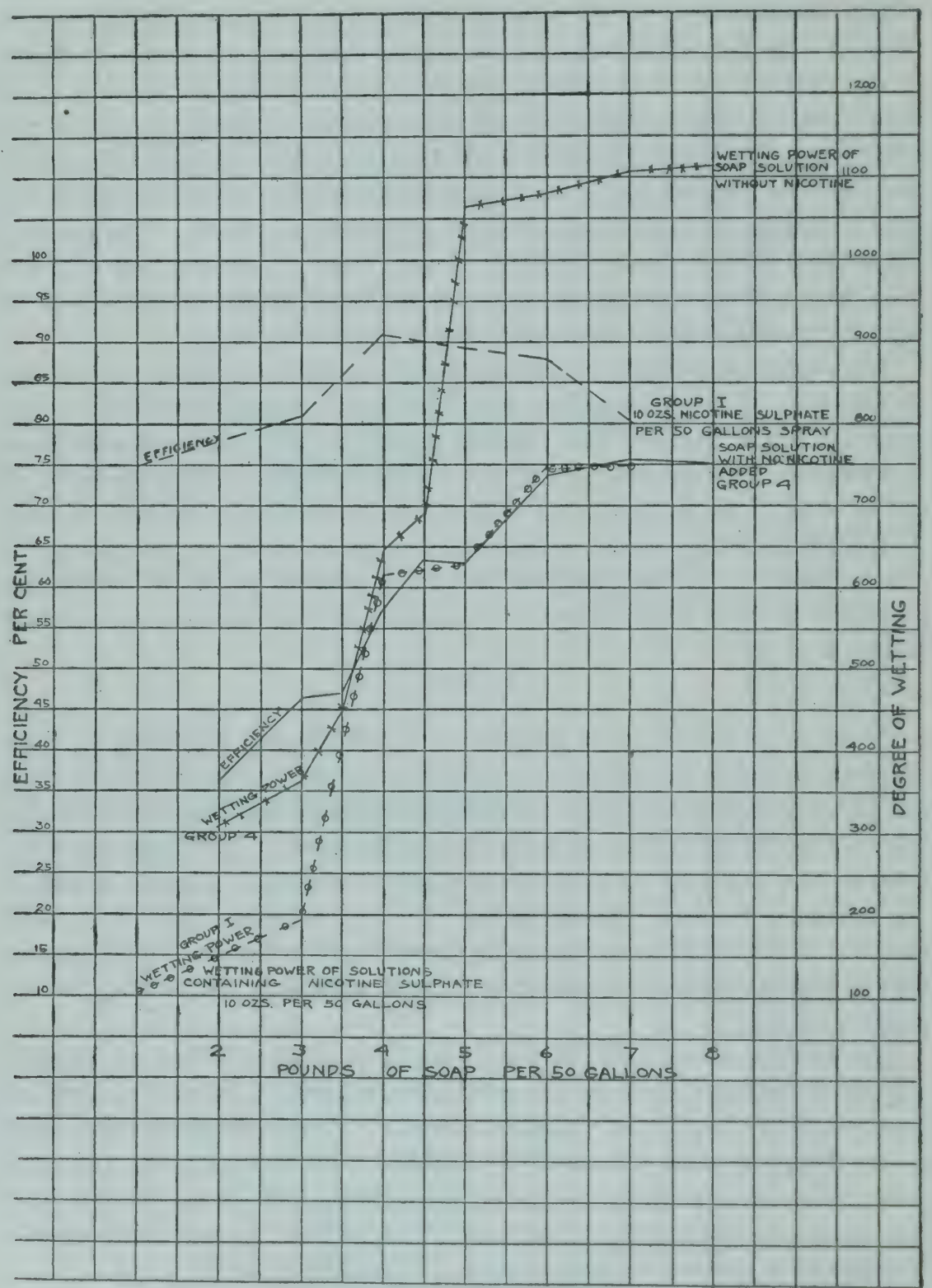

FIG. 1.—Efficiency and wetting power graphs for sprays in group 1, containing 10 ounces of nicotine sulphate and varying quantities of soap, and group 4, containing various amounts of soap with no nicotine. Wetting values are given in the column at the right.

(3) that if soap is used without the nicotine, 6 pounds to 50 gallons of water is all that can be used economically, since more concentrated solutions do not have an appreciably greater efficiency; (4) that when

nicotine sulphate at the rate of 1 to 630 is present in a soap solution the wetting power of this solution is less than that of one which contains an equal amount of soap without the nicotine; and (5) that when 4 pounds of soap or less are added to a 1-to-630 nicotine-sulphate solution the wetting power is but slightly affected by the presence of the nicotine. When more than 4 pounds of soap are added, the wetting powers of the subsequent solutions are greatly reduced from those of similar soap solutions containing no nicotine. The efficiency of the combination sprays likewise decreases from the point where the wetting power is influenced the least by the concentration.

From the foregoing statements it is evident that the addition of nicotine sulphate to soap solutions reduces the wetting power. A comparison of the wetting-power determinations of the soap solutions containing nicotine with those of the soap solutions without nicotine shows that the loss of wetting power is not by any means in direct ratio to the quantity of soap in the solution; therefore the loss is probably not entirely due to a physical effect of the nicotine upon the solution, for in that case the loss of wetting power would be proportional to the amount of soap contained in the solution before the nicotine was added. The wetting-power curves of the two groups of solutions indicate that a chemical change takes place when a certain degree of concentration is reached, which affects the physical properties of the solutions containing nicotine, and also that the effect is greater after a definite degree of concentration of soap is reached. Since all the sprays in group 1 have an efficiency of 75 per cent or more, depending on the amount of soap contained in the formula, and the highest efficiency of any of the sprays in group 4 was only slightly above 75 per cent, it is evident that the chemical reaction affects the soap and not the active nicotine sulphate. In support of this it may be stated that the solutions in groups 1, 2, and 3 were tested and it was found that the percentage of nicotine in them agreed with the amount of nicotine sulphate contained in the formulæ, and that the amount of soap present apparently had no influence upon the nicotine content of the solutions, for if any nicotine was set free by a reaction with the soap, less than 0.005 per cent was lost. This was not sufficient to cause any appreciable variation in their efficiency.

GROUPS 2 AND 3

The formulæ in group 2 contain 5 pounds of soap, which is constant for the group, plus varying quantities of nicotine sulphate to 50 gallons of water. Group 3 contains formulæ for 1 pound of soap, with similar amounts of nicotine sulphate as above, to 50 gallons of water. The efficiency and wetting-power curves for these groups appear in figure 2. The efficiency curve for group 2 rises gradually with the increased amounts of nicotine in the solution from 70 per cent at 3¼ ounces of nicotine to 93.5 per cent at 6½ ounces of nicotine in the solution. The efficiency of

8¾ ounces of nicotine was 95.9 per cent, the highest of any of the formulæ used in these experiments. This point of concentration is apparently the optimum, as the efficiency dropped to 86.8 per cent when 10 ounces of nicotine sulphate were used. The curve for the sprays in group 3 shows that the efficiency increases from 36.1 per cent for the formula containing 3¼ ounces to 69 per cent for the formula containing 6½ ounces of nicotine sulphate. From this point the efficiency rises gradually to 75 per cent for the formula containing 10 ounces of nicotine sulphate.

The wetting powers of the solutions in group 2 fall gradually from 788 for the formula containing 3¼ ounces to 732 for the formula containing 8¾ ounces of nicotine sulphate. Further concentration of the nicotine in the solution causes a considerable loss of wetting power, and the formula containing 10 ounces of nicotine has a wetting power of only 628. The wetting-power curve of the solutions in group 3 falls gradually from 181 for the formula containing 3¼ ounces to 103 for the formula containing 10 ounces of nicotine sulphate to 50 gallons of solution.

DISCUSSION OF GROUPS 2 AND 3

The main facts to be noted from the results given in figure 2 are: (1) The addition of 5 pounds of soap to the 50 gallons of nicotine solution increased the efficiency from 20 to 30 per cent more than that of similar nicotine solutions which contained only 1 pound of soap to 50 gallons of water; (2) the most efficient results were obtained with formulæ containing 5 pounds of soap, 6½ to 8¾ ounces of nicotine sulphate, and 50 gallons of water; (3) when more than 8¾ ounces of nicotine were added to the 5-to-50 soap solution there was a loss of efficiency and likewise a corresponding loss of wetting power; (4) while the quantities of soap in the solutions remained constant through both groups of formulæ, there was a gradual loss of wetting power, as the quantity of nicotine was increased in the solutions. The results derived from the formulæ in group 2 support the deductions already drawn from the results obtained with the formulæ in group 1—namely, that when certain concentrations of the soap and nicotine are reached, not only is there a decided loss of wetting power but there is also a corresponding loss in the insecticidal efficacy of the sprays. If nicotine sulphate is used at the rate of 1 to 630, the optimum efficiency is obtained with 4 pounds of soap to 50 gallons of water. By reducing the concentration of nicotine sulphate to 1 to 720, 5 pounds of soap to 50 gallons of solution gives the greater efficiency. The effect which a loss of wetting power may have upon the efficiency of a solution is indeterminable, since soap, as well as nicotine, has insecticidal properties. Thus, it is probable that a reaction which would cause a loss of wetting power would also reduce the insecticidal properties of the soap, ending in a loss of efficiency. There is

another condition which must be considered before judgment is made concerning the importance of wetting power. In case the insects are thoroughly drenched with the solution, a much higher percentage of

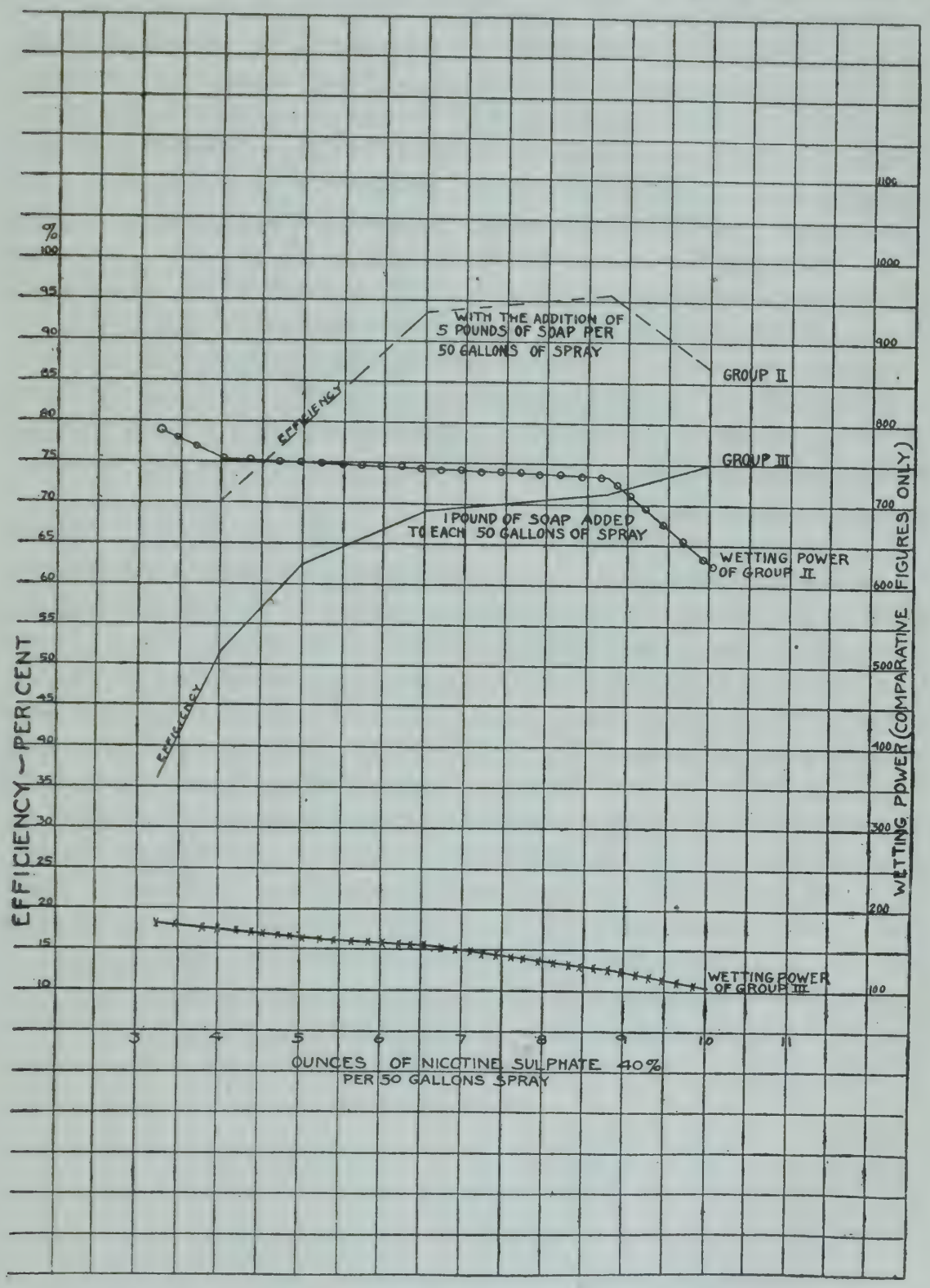
EFFICIENCY—PERICENT
WETTING POWER (COMPARATIVE FIGURES ONLY)
WITH THE ADDITION OF 5 POUNDS OF SOAP PER 50 GALLONS OF SPRAY
GROUP II
EFFICIENCY
GROUP III
1 POUND OF SOAP ADDED TO EACH 50 GALLONS OF SPRAY
WETTING POWER OF GROUP II
EFFICIENCY
WETTING POWER OF GROUP III
OUNCES OF NICOTINE SULPHATE 40% PER 50 GALLONS SPRAY

FIG. 2.—Efficiency and wetting-power gsaphs for group 2, containing 5 pounds of soap, and group 3, containing 1 pound of soap plus varying amounts of nicotine sulphate. Wetting values are in the column on the right.

mortality occurs than when the insects are struck by a few minute drops, as is usually the case in field spraying where the materials are applied at high pressure. Under the latter conditions wetting power is highly

important, for from our present knowledge the insects are killed by the absorption of the materials through the trachea, and in this case it would be necessary for the solution to spread over the body of the insect in order to gain entrance to the spiracles. A slight loss of wetting power of solutions which have an efficiency of 75 per cent or less would probably not cause an appreciable change in their effectiveness. This is shown in the case of group 3, in which the wetting power becomes less as the concentration of the solution is increased, but the efficiency rises in a normal curve. If a solution with an efficiency of 85 per cent or more upon further concentration loses wetting power, there is an appreciably greater corresponding loss of efficiency than would occur by increasing the concentrations of a solution whose efficiency is less than 75 per cent. The reason for this is obvious. A certain percentage of the insects are completely covered by the spray, so that the wetting-power influence on the efficiency is negligible; but when the insects which are struck by only a small quantity of the spray are considered, it is evident that the wetting power, as well as the strength of the solution, is an important factor governing its efficiency.

It is evident from the discussion in the preceding paragraphs that the loss of wetting power in the more concentrated mixtures is not due to a physical effect, but to chemical reactions caused by the nicotine sulphate in a soap solution. Since, if the nicotine, without causing chemical reactions, did exert an influence on the physical properties of the solutions, the loss of wetting power would be directly proportional to the amount of soap in the solution and also to the wetting powers of the soap solutions which contained no nicotine. Likewise, the loss of efficiency in the concentrated solutions is not due to a reaction which would cause a portion of the nicotine to be liberated as free nicotine, for the nicotine contents of the solutions were determined and the percentages were found to remain constant irrespective of the amounts of soap added to the solutions. From these facts we are led to assume that either a direct loss of wetting power or a reduction of the insecticidal value of the soap or both are contributing factors in the loss of efficiency of the more concentrated nicotine-sulphate and fish-oil-soap solutions.

SUMMARY

(1) When using combination fish-oil-soap and nicotine-sulphate sprays for the control of insects affecting truck crops, it was found that certain concentrated mixtures did not give as satisfactory results as did some of lower concentration. In connection with these results it was noticed during the spraying operations that some of the more concentrated solutions did not possess as high wetting or spreading powers as other mixtures which contained less soap.

(2) The spraying operations were performed on peas, spinach, and strawberries against the pea aphid, spinach aphid, and red spider,

respectively. The proportional efficiency of the sprays proved to be similar for each species. The efficiency of the sprays was determined by counting the number of live insects on a portion of the plot previous to the application of the sprays, and again determining the number two hours after treatment. The experiments were performed on 27 one-twentieth-acre plots. Peas were sprayed five times, spinach and strawberries once. The wetting powers as well as the nicotine content of the solutions were determined.

(3) When more than 4 pounds of soap were used with 10 ounces of nicotine sulphate to 50 gallons of water, there was a loss of both wetting power and efficiency.

(4) When more than 8¾ ounces of nicotine sulphate were combined with 5 pounds of fish-oil soap to 50 gallons, a loss occurred in both the wetting power and the efficiency.

(5) When nicotine sulphate was used in quantities up to 10 ounces, to a 1-to-50 fish-oil-soap solution, none of the resultant sprays had an efficiency of more than 75 per cent. Also, when fish-oil soap was used alone in quantities not exceeding 8 pounds to 50 gallons, the highest efficiency of any of the formulæ was only a fraction over 75 per cent.

(6) It was found that the nicotine content of the solutions remained the same irrespective of the amount of soap used.

(7) The loss of efficiency due to increasing the concentrations of the solutions is probably caused by a loss of both wetting power and insecticidal value of the soap.

(8) The loss of wetting power which occurs when the concentration of the solutions is increased has a stronger tendency to reduce the efficiency of the subsequent solutions, if the original solution has an efficiency of 85 per cent or more, than it does if the original efficiency is below 75 per cent.

(9) The actual importance of wetting power is difficult to determine in this case, as the fish-oil soap has insecticidal properties in itself. Where the wetting power is affected, it is probable that the soap is also broken down sufficiently to lose some of its value as an insecticide; hence, both factors must be considered as the cause of the loss of efficiency of some of the more concentrated mixtures.

LIFE HISTORY AND POISONOUS PROPERTIES OF CLAVICEPS PASPALI

By H. B. Brown, [1]

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INTRODUCTION

During the last decade *Paspalum dilatatum* Poir. has attained considerable prominence as a forage grass in various parts of the South. One serious objection to its use, however, is that forage poisoning frequently results among cattle feeding on it. Brown and Ranck [2] showed that the poisonous property is due to *Claviceps paspali* Stevens and Hall, a fungus that infects the grass very generally. This species was described by Stevens and Hall [3] in 1910. Norton [4] observed this fungus on *P. dilatatum* in Maryland in 1902. He suspected that it was poisonous, but carried on no feeding experiments to determine this.

Since September, 1914, the writer has been making a study of the life history of *Claviceps paspali* and its growth and distribution in the region about the Mississippi Agricultural College. In this region the fungus infects *Paspalum dilatatum* very generally, a few weeks after the grass heads out at least 90 per cent of the old heads showing infection.

LIFE HISTORY OF THE FUNGUS

Sclerotia produced during the summer and autumn (Pl. 32, F) drop to the ground when the old grass head sheds its spikelets, and lie on the ground until spring. They may be found at any time during the winter and spring by searching in the litter on the ground where infected *Paspalum dilatatum* grew the season before. Sclerotia gathered during the winter and placed in moist chambers kept at room temperature will germinate in 20 to 30 days, but it is the writer's experience that sclerotia forced in this way do not produce as many nor as large and vigorous stromata as those that germinate in the normal way. After a few days of rainy weather about the middle of May, sclerotia germinating on the ground may be expected. They were first found

[1] I wish to express my obligation to Dr. Charles F. Briscoe and to Prof. J. M. Beal, of the Mississippi Experiment Station, for the use of their laboratories in carrying on this work, and for other courtesies extended to me.

[2] Brown, H. B., and Ranck, E. M. Forage poisoning due to Claviceps paspali on Paspalum. Miss. Agr. Exp. Sta. Tech. Bul. no. 6, 35 p., 18 fig. 1915.

[3] Stevens, F. L., and Hall, J. G. Three interesting species of Claviceps. *In* Bot. Gaz., v. 50, no. 6, p. 460–463, 8 fig. 1910.

[4] Norton, J. B. S. Plant diseases in Maryland in 1902. *In* Rpt. Md. State Hort. Soc., v. 5, 1902, p. 90–99. [1902.]

on May 10 in 1915 and on May 21 in 1916. In each case this was just after the host plant had begun to flower.

The sclerotia of *Claviceps paspali* when mature are globular in shape, 2 to 4 mm. in diameter, irregularly roughened on the surface, and yellowish gray in color; the interior is homogeneous in structure and contains a considerable quantity of oil. Germinating sclerotia produce from one to several stromata, usually two or three, with slender whitish stalks 3 to 15 mm. in length, and heads about 1 mm. in diameter (Pl. 32, E). The heads are roughened over the surface owing to projecting perithecial necks (Pl. 32, A, E), and are at first whitish in color, later becoming rather bright yellow, and finally brownish.

A vertical section of a stromatic head (Pl. 32, A) shows numerous flask-shaped perithecia embedded in the outer part of the head. The neck of each perithecium projects a short distance beyond the surface, thus forming small pimple-like projections. Each perithecium contains numerous slender, cylindrical asci, 150 to 170μ in length (fig. 1, *a*); at the outer end of each ascus there is a thimble-like knob fitting over the end. The wall of the ascus is so thin that it can not be distinguished clearly. The ascospores are filiform and hyalin, being a little less than 1μ in diameter and 70 to 100μ in length (fig. 1, *c*). There are probably eight spores in an ascus, although not more than seven were counted with certainty. It was not possible to count the spores when inside an ascus, as they are hyalin and packed together closely, and it was a rather difficult matter to count them as the ascus disintegrated.

FIG. 1.—*Claviceps paspali*: *a*, Mature ascus; *b*, ascus breaking up to liberate spores; *c*, ascospore.

Mature stromatic heads from sclerotia just gathered from the field when allowed to dry slightly and then moistened exuded asci very freely. The asci go to pieces quickly after escaping from the perithecia and liberate the spores. A change of moisture conditions in the field will cause spores to be deposited on the surface of the stromatic head, where they are in position to be picked up by insects that chance to rub against the head. The stromata are somewhat tough and leathery and last for several days. If the ground becomes dry during their regular period they dry out, but revive with the coming of moisture and again shed spores. No stromata were found in the field after July 2.

Flowers of *Paspalum dilatatum* inoculated with ascospores by rubbing stromatic heads against stigmas and spikelets of the grass heads showed abundant evidence of infection in seven days. Flowers on control plants showed no infection. (Both inoculated plants and controls were kept under bell jars.) In the field, infected heads are not found for several days after the sclerotia germinate. They were first noticed on June 8 in

1915 and on June 12 in 1916, being, respectively, 29 and 22 days after germinating sclerotia were first found. In 1915, infected or diseased heads were not plentiful in the fields until about July 12. Preceding this date there were several days of rainy weather. In 1916, similar observations were made. Diseased heads became very common during July, following several weeks of rain. On August 1, 1916, they were more plentiful than since the autumn of 1914.

In the fields the first infection of the season is doubtless carried by insects. Running over the ground, they are likely to rub against the stromatic heads, which are covered with ascospores, and, climbing up the grass culms to take flight, may carry ascospores to the grass flowers and produce infection. That infection does not take place often is evidenced by the fact that the disease is slow in getting a start after the sclerotia germinate.

The infecting fungus attacks the pistil of the grass flower, and in a few days the ovary is almost entirely destroyed, a mass of fungus tissue filling the space it occupied. Plate 32, D, shows a section of the mass of fungus tissue between the glumes of a grass spikelet a week after infection. The two spots in the central part of the figure represent remnants of the grass flower. The rest of the central part of the section is homogeneous tissue, while around the edge are numerous tufts of hyphæ standing at right angles to the central mass. Figure C of Plate 32 shows the tufts enlarged. Each tuft contains a number of hyphæ. The digital ends of these hyphæ, or certain of them, enlarge and form conidia or sphacelia spores. Figure 2 shows the tip of a tuft of hyphæ. The spores are hyalin but show granules when stained, oblong, about 5μ wide and 15μ long. They are produced in great abundance and are carried from the hyphæ on which they were produced by a droplet of honeydew, a sticky, sweetish exudation of the fungus tissue. Insects of many kinds feed on this honeydew and carry infection by means of the spores clinging to their bodies. Hand inoculations, which were made by smearing honeydew containing sphacelia spores on flower stigmas, produced infections that were exuding honeydew and sphacelia spores freely within the space of a week. This result was obtained in the case of plants kept under bell jars, and also with plants inoculated in the field. Sphacelia spores frequently germinate in the droplet of honeydew and give it a whitish appearance.

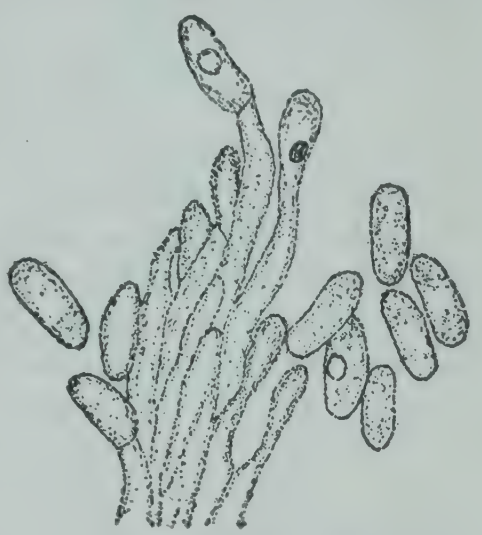

FIG. 2.—*Claviceps paspali*: Tip of tuft of hyphæ, showing the production of sphacelia spores.

The sphacelia stage in which honeydew is exuded lasts but a few days. If the weather is dry, the whole grass head is likely to become dry and dead, and no further development occurs. Or, again, honeydew may become infected with a species of Fusarium or Cladosporium and growth be stopped. If weather conditions are favorable, the solid mass of fungus tissue, constituting the bulk of the sphacelia tissue, continues to enlarge and soon forces the glumes of the spikelets apart. These masses are young sclerotia. In some cases within a week after the sphacelia stage was at its height the young sclerotia were projecting from between the glumes of the spikelet and were 1 to 2 mm. in diameter. Following this, some of the sclerotia continue to enlarge, attaining a maximum diameter of about 4 mm. and characters as outlined above. During September and October the largest sclerotia are to be found; sclerotia are also most plentiful then.

OTHER FUNGI INFECTING PASPALUM DILATATUM

As was mentioned above, *Fusarium heterosporum* Nees. and *Cladosporium* sp. are two other fungi found infecting heads of *Paspalum dilatatum*. While these fungi have not been studied carefully, they seem to be largely in the nature of molds growing on *Claviceps paspali* and parts of the diseased grass heads. The inoculation of healthy grass heads with spores from pure cultures of each of these fungi produced no infection. They are probably of no great consequence.

POISONOUS PROPERTIES OF CLAVICEPS PASPALI

As was shown by Brown and Ranck,[1] *Claviceps paspali* is poisonous to certain animals, especially to cattle and guinea pigs. It produces a peculiar nervousness, resembling considerably that shown in certain stages of rabies, and if eaten in quantity may cause death. A gram of extract made from this fungus, although probably containing other substances in addition to the poisonous element, will, if fed to a guinea pig, cause death within a few hours. Many cattle running on pastures in which the diseased grass is plentiful perish when under the influence of the poison by getting down in the pasture out of reach of water and feed. A good many others, too, perish by drowning in pools or ponds of shallow water. They fall into the water in a nervous paroxysm and drown before getting over it.

Guinea pigs used in feeding experiments showed nervousness after being fed 50 sclerotia that had been picked from old heads of *Paspalum dilatatum*. Continued feeding produced death within a week or less. In most cases the sclerotia were given in doses of 25 a day.

In feeding experiments carried on during the summer of 1915 it was found that sclerotia that had been in the laboratory for about 10 months

[1] Brown, H. B., and Ranck, E. M. Forage poisoning due to Claviceps paspali on Paspalum. Miss. Agr. Exp. Sta. Tech. Bul. 6, 35 p., 18 fig. 1915.

were still poisonous and that a small amount of the extract of *Claviceps paspali* exposed to air and hot summer temperature was still active after a period of about 10 months.

A guinea pig fed 40 grass spikelets daily for seven days, each containing a mass of *Claviceps paspali* tissue in the sphacelia, or honeydew, stage, and 60 each day for the next 36 days, showed no bad effects, but gained in weight. Another pig fed 25 young sclerotia daily for 7 days and 40 daily for the next 21 days showed no bad effects, but gained in weight slightly. This feeding was started on July 16. The last two experiments seem to indicate that it is only the old sclerotia that are poisonous. The experience of farmers with cattle on pastures indicates the same.

Mowing pastures one or more times during the late summer or autumn, or as often as mature sclerotia become abundant, is an effective method of preventing poisoning, and is a measure of practical value in most places.

PLATE 32

A.—Section through a mature stromatic head of *Claviceps paspali*, showing perithecia containing asci. ×45.

B.—Spike of *Paspalum dilatatum* with mature sclerotia attached. Nearly natural size.

C.—Tufts of hyphæ producing sphacelial spores. ×150.

D.—Section of mass of tissue within grass spikelet during sphacelia stage of *Claviceps paspali;* spores are produced by tufts of hyphæ along edge of section. ×50.

E.—Sclerotium of *Claviceps paspali* with stromata. ×5.

F.—Spikes of *Paspalum dilatatum*, showing a number of sclerotia attached. About one-half natural size.

A
B
C
D
E
F

EFFECT OF SODIUM SALTS IN WATER CULTURES ON THE ABSORPTION OF PLANT FOOD BY WHEAT SEEDLINGS

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INTRODUCTION

The following experiments were undertaken to determine the extent to which the presence of the various sodium salts commonly found in alkali soils affects the absorption of plant-food elements by wheat seedlings. Sodium chlorid, sodium sulphate, and sodium carbonate in concentrations ranging from 50 to 1,000 p. p. m.[1] were employed in connection with a standard nutrient solution, consisting of 200 p. p. m. of NO_3 as sodium nitrate, 200 p. p. m. of K_2O as potassium chlorid, and 130 p. p. m. of P_2O_5 as sodium phosphate, together with calcium carbonate ($CaCO_3$) in excess. The same variety of hard wheat, Minnesota Bluestem C. I. 169 (*Triticum vulgare*), was used in all the measurements.

CULTURE METHOD

The enameled culture pans each contained 2,500 c. c. of the nutrient solution. Each pan was provided with a perforated aluminum disk, supported on sealed glass buoys, so as to float at the surface of the solution. Wheat seeds were sprinkled over the disks in numbers sufficient to provide about 1,000 seedlings in each pan.

The nutrient solution in each pan was changed every two days and during the intervening period was kept approximately at the original volume by the addition of water. The analyses showed that with this method of procedure there was always an abundance of plant food at the disposal of the seedlings.

During the first two days of the experiment the seedlings were grown in the nutrient solution alone. At the end of the second day sodium chlorid, sodium sulphate, and sodium carbonate were added to the nutrient solution in concentrations varying from 50 to 1,000 p. p. m., as shown in Tables I to IV. The sodium carbonate in the lower concentrations gradually changed to sodium bicarbonate, owing to the absorption of carbon dioxid from the atmosphere and to its evolution from the roots of the growing seedlings. Where the original concentration was 300 p. p. m. and above, sodium carbonate was still present after the plants had been grown in the culture solution for two days.

[1] Parts per million in solution by weight.

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The cultures were carried on for 21 days in the sunshine at Riverside, Cal. At the end of this period the green and dry weights of 100 representative plants from each culture pan were determined, and the plants were analyzed by official methods for nitrogen, phosphoric acid, and potash. The results are given in Tables I, II, III, and IV, the weight in each instance being based upon 100 plants.

SODIUM CHLORID

The experimental data obtained with nutrient solutions containing graduated amounts of sodium chlorid are given in Table I, and in figure 1 the quantities of potash, phosphoric acid, and nitrogen contained in 100 plants are plotted separately against the sodium-chlorid concentration. The presence of sodium chlorid in the nutrient solution appears to

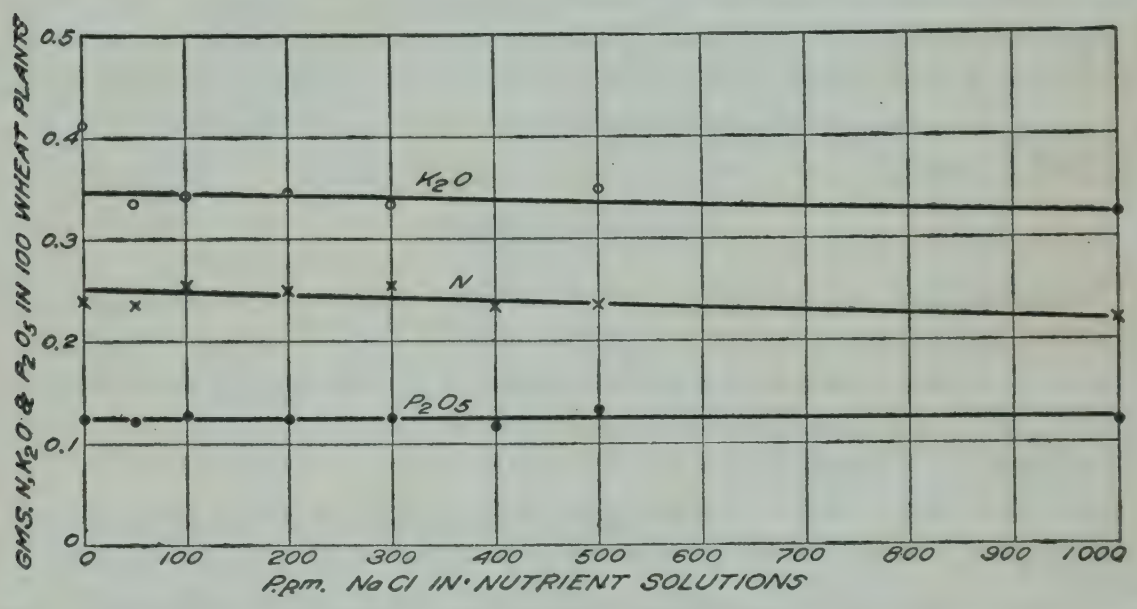

FIG. 1.—Graphs showing the effect of sodium chlorid in nutrient solutions on the nitrogen, potash, and phosphoric-acid content of wheat seedlings.

diminish very slightly the potash and nitrogen content of the wheat plants, but the effect is so small as to be comparable with experimental errors. The phosphoric-acid content of the wheat seedlings appears to be quite independent of the amount of sodium chlorid in the culture solution for the range in concentration here employed.

The nitrogen, potash, and phosphoric-acid content of the seedling wheat plants grown in the presence of sodium chlorid are also expressed in Table I in percentage of the dry weight of the plants. The results in this form are not as concordant as those already discussed, but lead to the same conclusion—namely, that the presence of sodium chlorid in culture solutions in graduated concentrations up to 1,000 p. p. m. has very little effect upon the total nitrogen, potash, and phosphoric-acid content of young wheat plants.

TABLE I.—*Effect of sodium chlorid on the weight and composition of wheat seedlings*

Culture No.	Parts per million of sodium chlorid added to nutrient solution.	Green weight of 100 plants.	Dry weight of 100 plants.	Weight of element in 100 plants.			Percentage of dry weight of plants.		
				N.	K_2O.	P_2O_5.	N.	K_2O.	P_2O_5.
		Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*			
1	0	71.5	6.21	0.240	0.411	0.125	3.9	6.6	2.0
2	50	59.5	5.70	.236	.334	.119	4.1	5.9	2.1
3	100	58.5	5.70	.252	.343	.125	4.4	6.0	2.2
4	200	61.5	5.70	.249	.345	.124	4.4	6.1	2.2
5	300	63.5	5.70	.251	.332	.125	4.4	5.8	2.2
6	400	64.5	5.96	.230	.288	.115	3.9	4.8	1.9
7	500	59.0	5.29	.231	.348	.133	4.4	6.6	2.5
8	1,000	56.6	5.55	.216	.322	.121	3.9	5.8	2.2

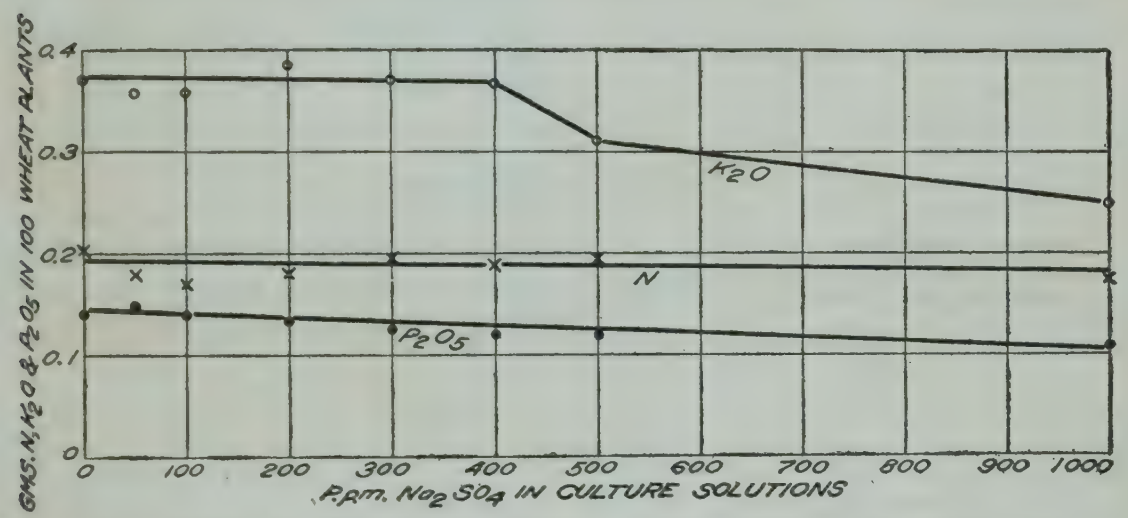

FIG. 2.—Graphs showing the effect of sodium sulphate in nutrient solutions on the nitrogen, potash, and phosphoric-acid content of wheat seedlings.

SODIUM SULPHATE

The data obtained with culture solutions containing graduated amounts of sodium sulphate are given in Table II. The results of the analyses of the plants for nitrogen, potash, and phosphoric acid are given both in terms of the actual amounts found in 100 plants from each culture and also in percentage of the dry weight of the plants. The dry weight is nearly uniform, so that the percentage relationship does not differ materially from that represented by the absolute amounts of nitrogen, potash, and phosphoric acid found. The latter determinations are plotted as ordinates in figure 2 and the concentration of sodium sulphate in the culture solutions as abscissæ. The results show that the addition of sodium sulphate to the nutrient solution in concentrations up to 1,000 p. p. m. has practically no effect on the total nitrogen content of the young wheat plants. In concentrations greater than 400 p. p. m. the sodium sulphate depressed the potash content slightly. In the case of phosphoric acid the plants show a very slight but steady decrease in the

total phosphoric-acid content as the concentration of the sodium sulphate increases. This effect is in evidence throughout the range of sodium-sulphate concentrations employed.

TABLE II.—*Effect of sodium sulphate on the weight and composition of wheat seedlings*

Culture No.	Parts per million of sodium sulphate added to nutrient solution.	Green weight of 100 plants.	Dry weight of 100 plants.	Weight of element in 100 plants.			Percentage of dry weight of plants.		
				N.	K_2O.	P_2O_5.	N.	K_2O.	P_2O_5.
		Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*			
1	0	63.1	5.85	0.206	0.369	0.141	3.5	6.3	2.4
2	50	67.0	6.07	.178	.367	.150	2.9	6.0	2.4
3	100	69.4	6.23	.163	.367	.140	2.6	5.9	2.2
4	200	66.6	5.75	.181	.385	.133	3.1	6.7	2.3
5	300	67.2	6.08	.194	.370	.125	3.2	6.1	2.1
6	400	65.5	6.13	.184	.367	.120	3.0	6.0	2.0
7	500	62.5	5.75	.194	.311	.120	3.4	5.4	2.1
8	1,000	59.0	5.75	.176	.248	.106	3.1	4.3	1.8

SODIUM CARBONATE

The data obtained with culture solutions containing sodium carbonate are presented in Table III. The absolute quantities of potash, nitrogen, and phosphoric acid found in 100 seedlings are plotted in figure 3 against

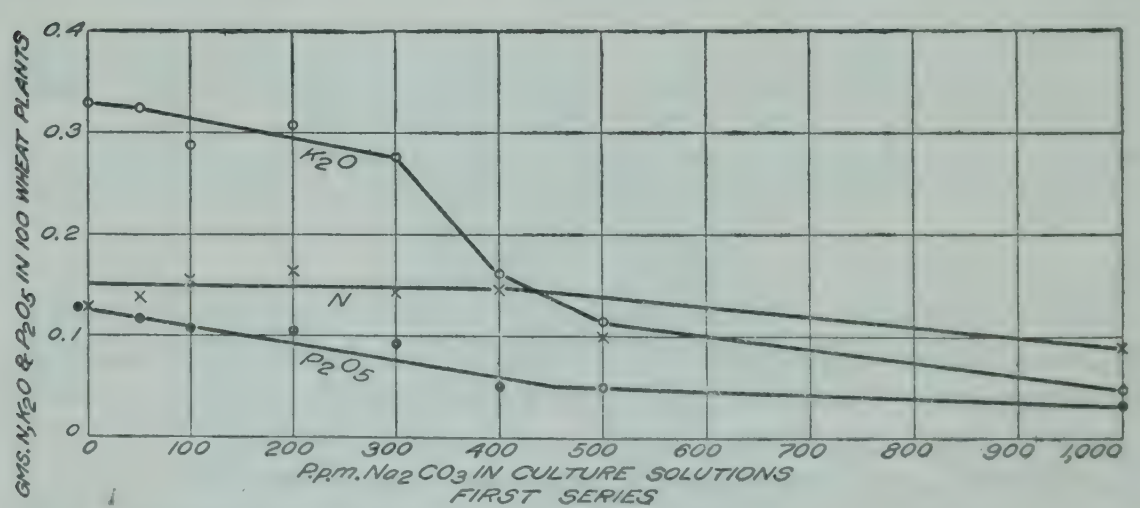

FIG. 3.—Graphs showing the effect of sodium carbonate on the nitrogen, potash, and phosphoric-acid content of wheat seedlings. First series.

the sodium-carbonate concentration. The results show a marked reduction in the amount of potash and phosphoric acid in the seedlings as the concentration of the sodium carbonate increases, the total potash or phosphoric-acid content of 100 wheat plants grown in the presence of 1,000 p. p. m. of sodium carbonate being only one-third that of the plants grown in the control solutions. The total nitrogen content of the wheat plants is also slightly decreased.

TABLE III.—*Effect of sodium carbonate on the weight and composition of wheat seedlings*

FIRST SERIES

Culture No.	Parts per million of sodium carbonate added to nutrient solution.	Green weight of 100 plants.	Dry weight of 100 plants.	Weight of element in 100 plants.			Percentage of dry weight of plants.		
				N.	K_2O.	P_2O_5.	N.	K_2O.	P_2O_5.
		Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*			
1	0	60.3	5.59	0.128	0.329	0.130	2.3	5.8	2.3
2	50	61.0	5.65	.139	.327	.118	2.5	5.8	2.1
3	100	53.6	4.89	.156	.287	.108	3.2	5.9	2.2
4	200	53.2	4.93	.164	.307	.105	3.3	6.2	2.1
5	300	50.5	4.92	.140	.275	.093	2.9	5.6	1.9
6	400	32.7	3.36	.148	.158	.050	4.4	4.7	1.5
7	500	28.0	3.81	.098	.113	.049	2.6	2.9	1.3
8	1,000	15.5	2.77	.087	.047	.037	3.2	1.7	1.3

SECOND SERIES

1	0	71.5	6.21	0.240	0.411	0.125	3.9	6.6	2.0
2	50	57.5	5.61	.230	.332	.115	4.1	5.9	2.1
3	100	57.5	5.42	.257	.331	.116	4.7	6.1	2.1
4	200	52.5	5.11	.209	.359	.101	4.1	7.0	2.0
5	300	40.5	4.42	.160	.291	.097	3.6	6.6	2.2
6	400	31.8	4.25	.125	.230	.067	2.9	5.4	1.6
7	500	36.5	4.11	.144	.144	.057	3.5	3.5	1.4
8	1,000	20.5	3.16	.110	.061	.046	3.5	1.9	1.5

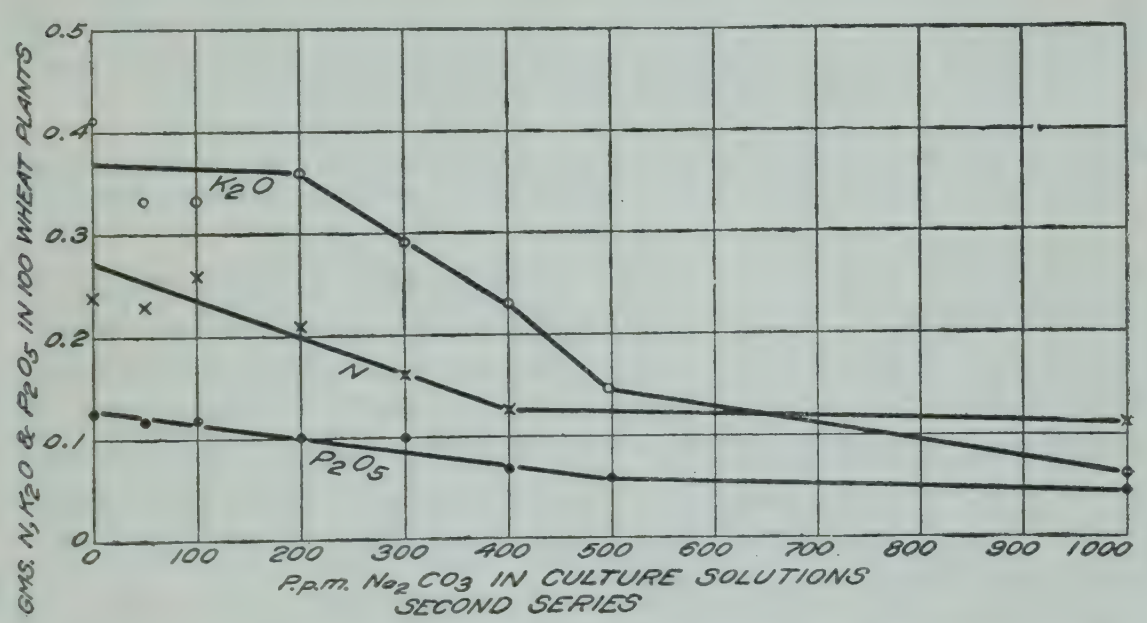

FIG. 4.—Graphs showing the effect of sodium carbonate on the nitrogen, potash, and phosphoric-acid content of wheat seedlings. Second series.

These results are in such striking contrast with the effects obtained with the other sodium salts that the sodium-carbonate series was repeated. The results of the second series of determinations are also given in Table III and are presented graphically in figure 4. A marked reduc-

tion in the potash and phosphoric-acid content is again shown as the sodium-carbonate concentration increases. In this series also there is a decided reduction in the total nitrogen content with increasing concentration of the sodium carbonate.

Reference to Table III will show that the weight of the seedlings decreased markedly as the concentration of the sodium carbonate increased. It is consequently of interest to express the nitrogen, potash,

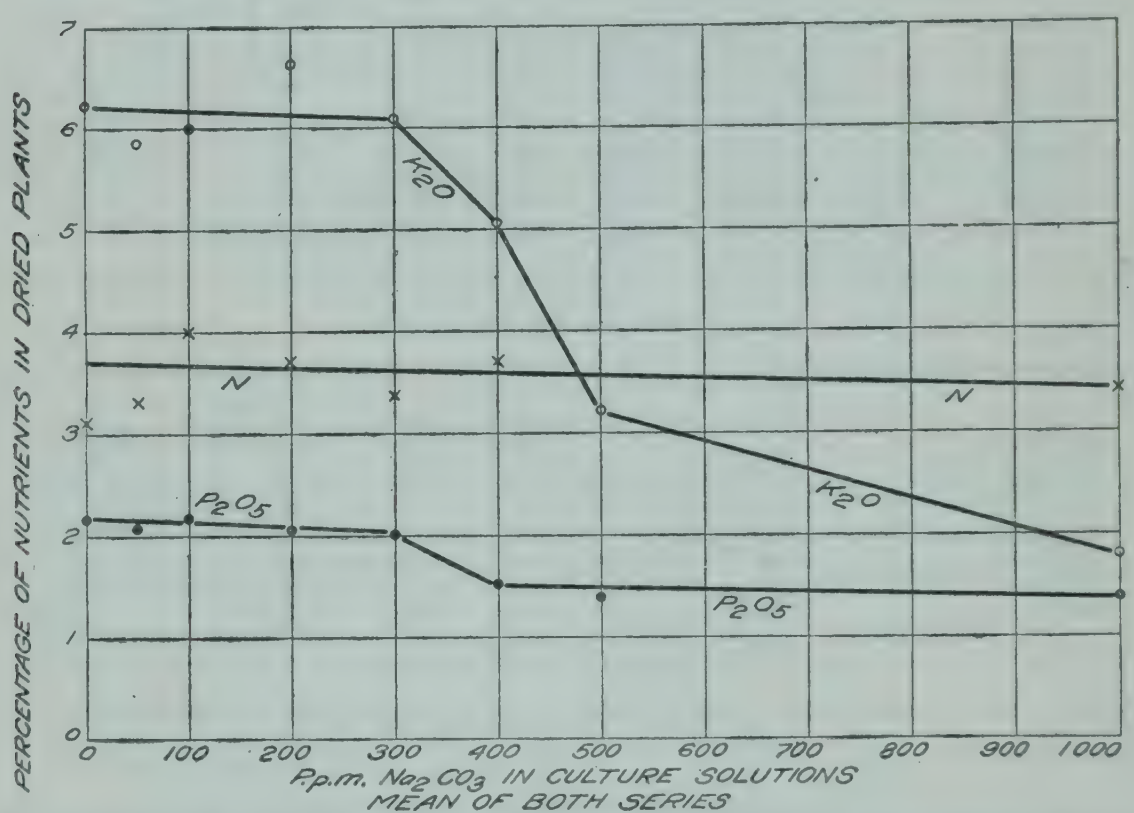

FIG. 5.—Graphs of the mean values of the first and second series showing the effect of sodium carbonate on the nitrogen, potash, and phosphoric-acid content expressed in percentage of the dry weight of wheat seedlings.

and phosphoric-acid content of the seedling plants in percentage of their dry weight. The results computed on this basis will be found in the last three columns of the table. The mean values for both series of determinations are plotted in figure 5. It will be seen that the percentage of nitrogen does not show any consistent change as the concentration of the sodium carbonate increases. The percentages of potash and phosphoric acid, on the other hand, decrease markedly with increasing concentration of the sodium carbonate.

NITROGEN, POTASH, AND PHOSPHORIC ACID ABSORBED FROM THE NUTRIENT SOLUTION BY THE VARIOUS CULTURES

The data so far given include the nitrogen, potash, and phosphoric acid stored in the seed. The analysis of 100 seeds for these substances gave the following results:

	Gram.
Weight of 100 seeds (dry)	2.45
Nitrogen	.0486
Potash (K_2O)	.0185
Phosphoric acid (P_2O_5)	.0242

If it is assumed that each lot of 100 seeds is of uniform composition and weight, the amount of nitrogen, potash, and phosphoric acid absorbed from the nutrient solutions by the various cultures can be determined by deducting the above quantities from those found in the plants grown in the culture solutions. The data obtained from the various cultures, reduced to this basis, are given in Tables V and VI, and are presented graphically in figures 6 to 8, the last figure representing the mean of the two sodium-carbonate series.

TABLE V.—*Effect of sodium chlorid and sodium sulphate on the absorption of nutrients by wheat seedlings*

Culture No.	Sodium chlorid.				Sodium sulphate.			
	Sodium chlorid added to nutrient solution.	Elements absorbed from solution (in percentage of dry weight of plants).			Sodium sulphate added to nutrient solution.	Elements absorbed from solution (in percentage of dry weight of plants).		
		N.	K_2O.	P_2O_5.		N.	K_2O.	P_2O_5.
	P. p. m.				*P. p. m.*			
1	0	3.1	6.3	1.7	0	2.8	6.0	2.0
2	50	3.3	5.5	1.7	50	2.1	5.7	2.1
3	100	3.6	5.7	1.9	100	1.8	5.6	1.9
4	200	3.5	5.9	1.8	200	2.3	6.4	1.9
5	300	3.6	5.5	1.9	300	2.4	5.8	1.8
6	400	3.0	4.5	1.5	400	2.2	5.7	1.6
7	500	3.5	6.6	2.1	500	2.5	5.1	1.7
8	1,000	3.0	5.5	1.7	1,000	2.2	4.0	1.4

The percentage of nitrogen absorbed by the young wheat plants does not appear to be measurably modified by the presence of any of the sodium salts investigated in concentrations up to 1,000 p. p. m. Sodium chlorid in this concentration does not affect the absorption of phosphoric acid measurably (fig. 6), but depresses slightly the percentage of potash absorbed. Sodium sulphate depresses the absorption of potash decidedly and of phosphoric acid slightly (fig. 7), while with sodium carbonate the depression in the absorption of both potash and phosphoric acid is very marked (fig. 8). The depressing effect of sodium carbonate on the

absorption of potash and phosphoric acid is in evidence even in concentrations of sodium carbonate as low as 100 p. p. m. It is evident

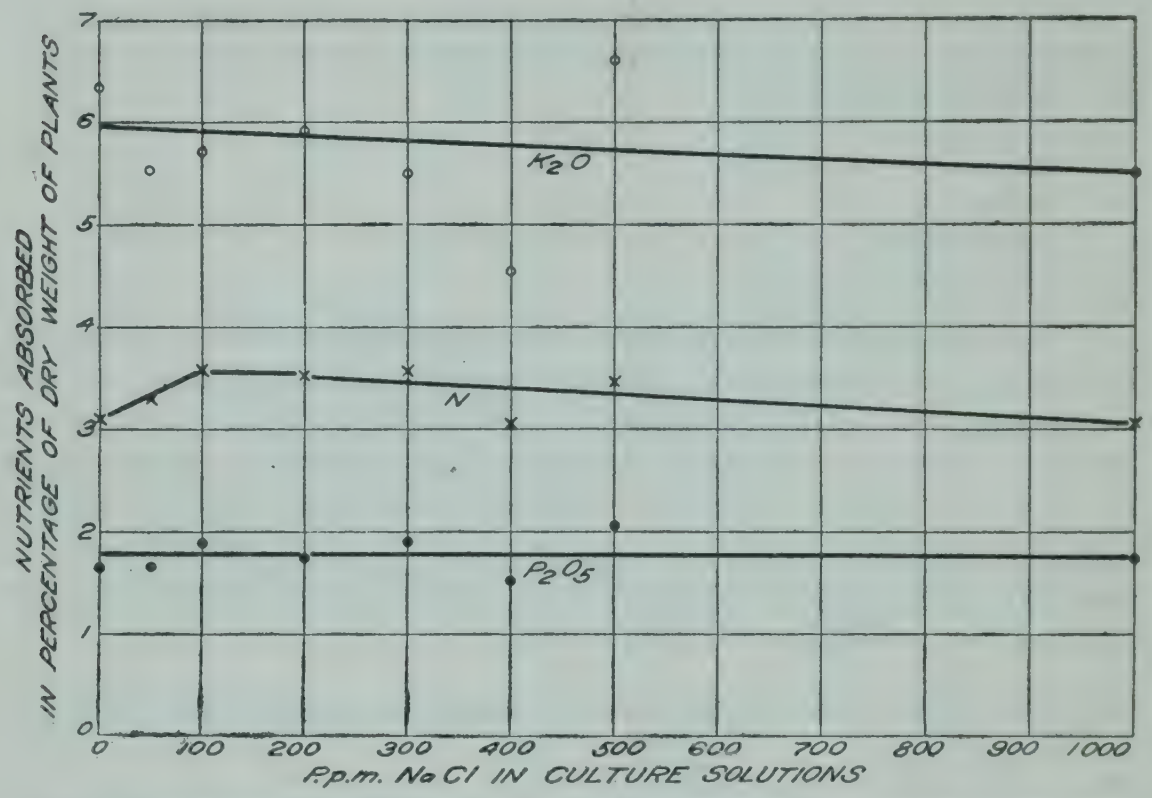

FIG. 6.—Graphs showing the effect of sodium chlorid on the absorption of nutrients by wheat seedlings.

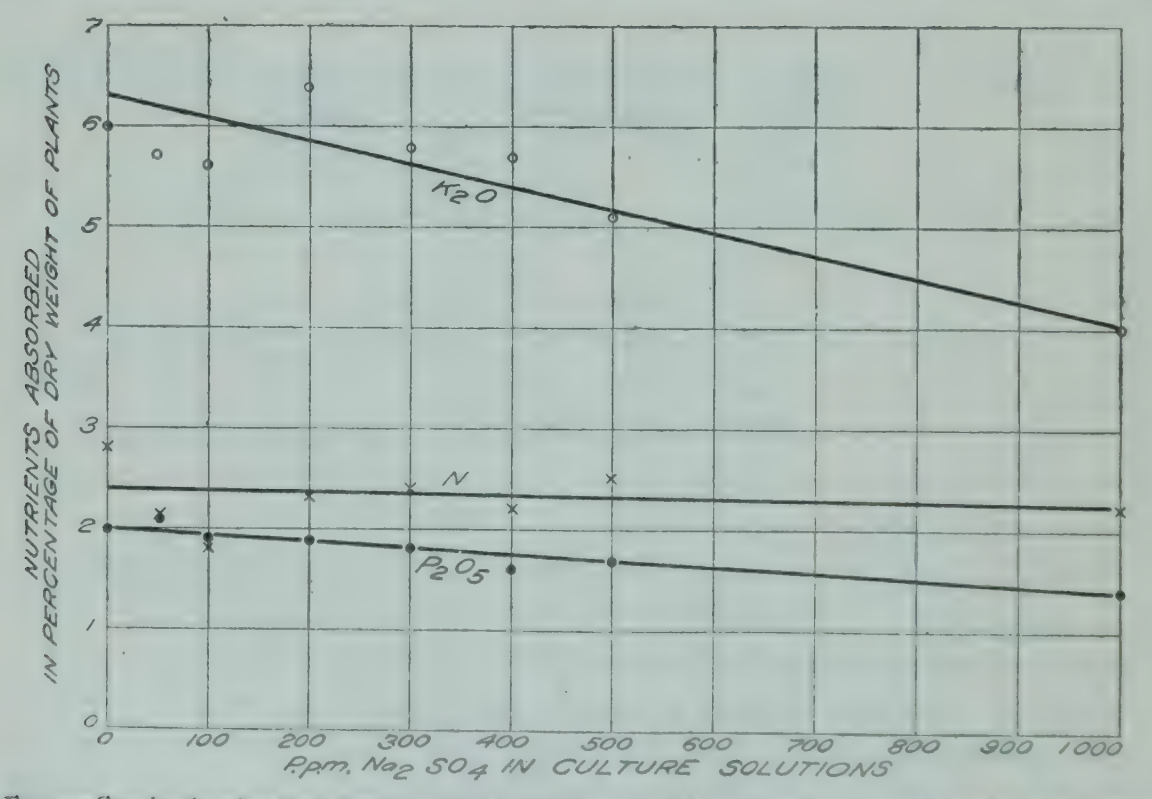

FIG. 7.—Graphs showing the effect of sodium sulphate on the absorption of nutrients by wheat seedlings.

from these measurements that the presence of sodium carbonate, even in these minute amounts, may have a markedly deleterious effect upon the metabolism of the small-grain crops.

TABLE VI.—*Effect of sodium carbonate on the absorption of nutrients by wheat seedlings*

FIRST SERIES

Culture No.	Sodium carbonate added to nutrient solution.	Elements absorbed from solution (in percentage of dry weight of plants).		
		N.	K_2O.	P_2O_5.
	P. p. m.			
1	0	1.4	5.6	1.9
2	50	1.6	5.6	1.7
3	100	2.2	5.5	1.7
4	200	2.3	5.9	1.6
5	300	1.9	5.2	1.4
6	400	3.0	4.2	.8
7	500	1.3	2.5	.7
8	1,000	1.4	1.0	.4

SECOND SERIES

1	0	3.1	6.3	1.7
2	50	3.2	5.6	1.6
3	100	3.9	5.8	1.7
4	200	3.1	4.7	1.5
5	300	2.5	6.2	1.6
6	400	2.0	5.0	1.0
7	500	2.3	3.0	.8
8	1,000	1.9	1.3	.7

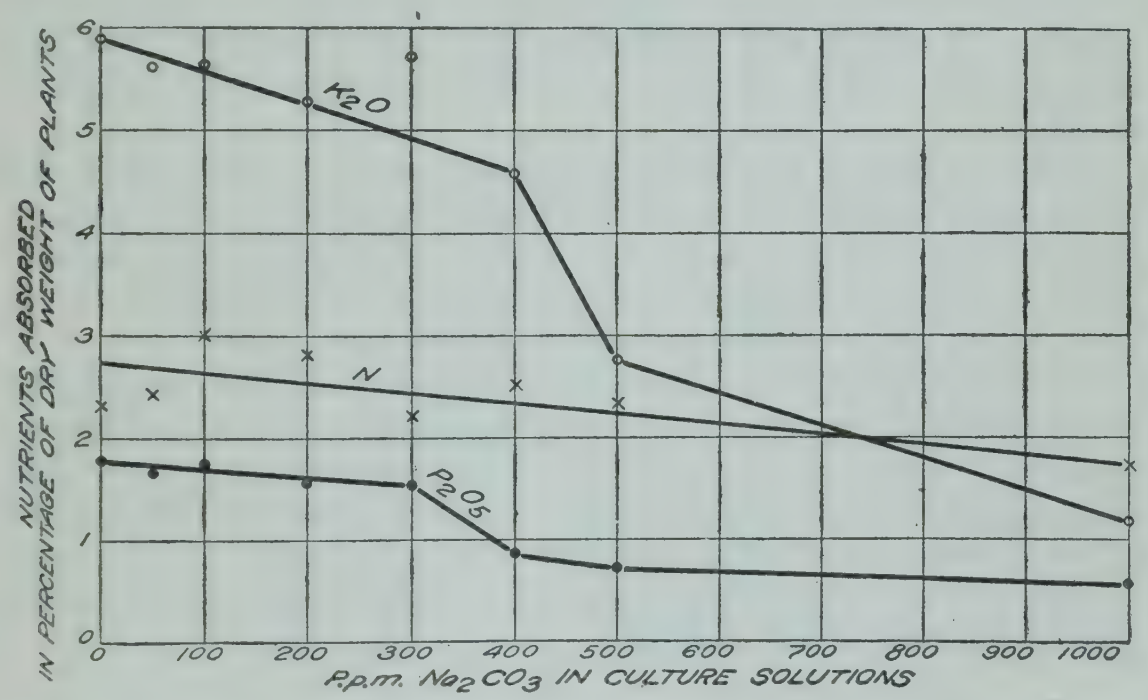

FIG. 8.—Graphs showing the effect of sodium carbonate on the absorption of nutrients by wheat seedlings.

It has been shown by Le Clerc and Breazeale[1] that there is a very marked absorption of potash by sprouting grain seedlings. As potash appears to be vitally concerned in the decomposition and translocation

[1] Le Clerc, J. A., and Breazeale, J. F. Translocation of plant food and elaboration of organic plant material in wheat seedlings. U. S. Dept. Agr. Bur. Chem. Bul. 138, 32 p., 2 fig. 1911.

of carbohydrates, any salt such as sodium carbonate, which would interfere with the absorption of potash at this stage of growth would seriously handicap the development of the plant.

It will be recalled that calcium carbonate was present in the culture solutions in the solid phase. It will be shown in another paper that appreciable quantities of sodium carbonate are formed through the reaction of sodium chlorid and sodium sulphate with calcium carbonate, and the resulting hydrolysis is greater with sodium sulphate than with sodium chlorid. This is in harmony with the greater activity shown by sodium sulphate in depressing the absorption of potash and phosphoric acid and suggests that the effect observed in the case of sodium chlorid and sodium sulphate may be in part due to the small amounts of sodium carbonate formed through reaction with the calcium carbonate.

CONCLUSIONS

Sodium chlorid, sodium sulphate, and sodium carbonate added to nutrient solutions in concentrations up to 1,000 p. p. m. do not measurably affect the nitrogen absorbed from culture solutions by young wheat plants.

Sodium chlorid in concentrations up to 1,000 p. p. m. does not affect the absorption of phosphoric acid, but decreases slightly the absorption of potash.

Sodium sulphate in concentrations of 1,000 p. p. m. depresses the absorption of potash and phosphoric acid to approximately 70 per cent of that of the control cultures, expressed in percentage of dry weight of the plants.

Sodium carbonate in concentrations of 1,000 p. p. m. reduces the absorption of potash to 20 per cent of that of the control and the absorption of phosphoric acid to 30 per cent of that of the control. The depressing effect of sodium carbonate is in evidence in concentrations as low as 100 p. p. m., and is marked in concentration of 300 p. p. m.

The relative effect of sodium sulphate and sodium chlorid in depressing the absorption of potash is directionally the same as the relative hydrolysis resulting from the reaction of the two salts with the calcium carbonate present in the culture solution. This suggests that the observed effects in the case of sodium sulphate and sodium chlorid may be due in part to the accumulative action of the slight amounts of sodium carbonate formed in this reaction.

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NITRIFICATION IN SEMIARID SOILS—I.[1]

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HISTORICAL INTRODUCTION

The distribution and amounts of nitrogen in the humus of the arid and semiarid soils of America and the activities of the microorganisms contained therein have been discussed at length by a number of writers. It has been especially emphasized that, as a rule, the humus and nitrogen are distributed more uniformly to a greater depth in the subsoil of the arid than of the humid regions. The soils are commonly very deep and are not sharply separated from the subsoils, but the actual percentage of nitrogen in the surface soil is frequently low and usually decreases in passing downward into the subsoil. The soils and subsoils in many parts of California, for example, contain less than 0.05 per cent of nitrogen and in many localities even less than 0.03 per cent.

Hilgard (10),[3] Loughridge (26), Lipman (16), and others have directed attention to the great depth of root penetration in the semiarid region, and Loughridge has suggested that the apparent deficiency of nitrogen in the surface soils may be compensated for by the distribution of nitrogen in the deep subsoils. He finds, for example, that the total nitrogen in the zone occupied by plant roots commonly compares favorably with that in humid regions. But, as is well known, the application of nitrogenous fertilizers commonly results in marked stimulation to crops, and in many localities successful crop production depends upon the use of nitrogenous fertilizers. As much as 1,000 to 1,500 pounds of dried blood per acre has been applied annually to some of the Citrus groves of southern California, and in some cases as much as 2,000 pounds per acre has been applied. Corresponding amounts of other nitrogenous materials have been used.

[1] Paper 35, Citrus Experiment Station, College of Agriculture, University of California, Riverside, Cal.
[2] The writer acknowledges the valuable analytical assistance of Mr. A. B. Cummins.
[3] Reference is made by number to "Literature cited," p. 436–437.

In the course of investigations on the origin of the so-called "niter spots" in Colorado, Sackett (28) concluded that nitrification is unusually active in Colorado soils and more so than in soils from certain other Western States. Stewart, Greaves, and coworkers (31, 32, 33, 34) in Utah concluded, on the other hand, that the excessive accumulation of nitrates in the surface soils of certain localities is to be accounted for mainly by the capillary rise of nitrates from deeper strata rather than from unusual nitrifying activity in the soils at the present time.

Lipman (16), by the use of the modified Remy solution method, found that ammonification and nitrification are most active in the first foot of California soils, but in some cases these processes were also found to take place quite actively in the subsoils down to a depth of 8 to 10 feet. He concluded that the deep penetration of the roots of cultivated plants may be accounted for in part by the active formation of available nitrogen in the deep substrata. McBeth and Smith (27) also found from experiments with the use of actual soil as the culture medium that dried blood and ammonium sulphate undergo active nitrification in the first foot of certain Utah soils, but the activity was found to decrease markedly in the successive sections below the first foot until at a depth of 4 or more feet it almost ceased. In their experiments about 90 per cent of the total nitrate produced in the 5-foot sections studied was formed in the first foot, from which it would seem that nitrification is not particularly active in the substrata below the first foot.

Greaves (8) found that, as a rule, cultivation brings about an increase in the numbers of organisms and in the rates of nitrification and nitrogen fixation in certain soils of Utah; he has also studied the effects of different arsenic compounds on the biological activities in soils (7, 9). Lipman and Burgess (17, 18, 19, 22, 23) have devoted considerable study to the effects of alkali salts and small amounts of copper, zinc, iron, and lead compounds on ammonification and nitrification in California soils.

Recently Lipman and Burgess (24) published experiments on the rates of nitrification of different fertilizers in 29 different soils from California. Briefly, it was found that, on the whole, ammonium sulphate was most actively and quite vigorously nitrified in most of the soils studied. The rates at which the different organic substances underwent nitrification varied widely. In certain soils low in organic matter little or no nitrate was formed from dried blood or high-grade tankage, while at the same time cottonseed meal, bone meal, garbage tankage, and other low-nitrogen-containing materials were quite vigorously nitrified. They concluded as follows:

> In all soils of our interior arid valleys which are not very close to stream channels or those which for other reasons are markedly deficient in organic matter, the proper bacteriological and perhaps other conditions do not obtain to render into nitrates most economically and quickly the nitrogen of high-grade organic nitrogenous fertilizers. On the other hand, conditions in those same soils are much more favorable for the nitrification of nitrogen of the low grade nitrogenous fertilizers. Similar con-

ditions prevail in the humus-poor soils of our coast valleys and of other valleys in the state, in which either the soils have always been deficient in organic matter or have become depleted in that respect through excessive oxidation under favorable climatic conditions assisted by constant summer cultivation.

In connection with the above and other investigations, Lipman has found that ammonification is active in California soils generally and that in some cases greater amounts of ammonia than nitrate occur in soil samples freshly drawn from the field. This condition was especially noted in soils on which the plant diseases known as dieback and mottle-leaf of *Citrus* spp. occur. In these soils Lipman (20, 21) found nitrification to be inactive. He suggested a causal relationship between the active ammonification and inactive nitrification on the one hand and the abnormal plant growth on the other, the latter being attributed to enforced ammonia absorption occasioned by the inability of the soil to transform ammonium nitrogen into nitrates.

As contrasted with the above hypothesis, Kellerman and Wright (11) have pointed out that the physiological disease known as mottle-leaf of Citrus trees may be caused by excessive accumulations of nitrates in the soil; but the source of the nitrate, whether being actively formed at the present time or otherwise, is not clear from their publication.

Beckwith, Vass, and Robinson (3) have studied the effects of lime on the ammonification of dried blood and peptone, and the nitrification of dried blood and ammonium sulphate in six soils from Oregon. In every case they found that active ammonification took place, but in two of the soils less nitrate was found after four weeks' incubation where either dried blood or ammonium sulphate had been added than in the portions to which no nitrogenous material was added. In one case the further addition of lime failed to induce the nitrification of these materials. An increase in the nitrate content in the check portions was found in every case, indicating the presence of the nitrifying organisms in the soil.

From the foregoing partial review of the literature on this subject it is apparent that radical differences of opinion are held with reference both to the formation and the movement of nitrates in the soils of the semiarid region.

In view of the economic importance of nitrogen and the scientific interest attached to nitrification, the writer has for some time been engaged in a series of studies on this subject at the University of California Citrus Experiment Station, at Riverside, Cal. At this place a fertilizer experiment with Citrus trees has been maintained during the past nine years. The plots of this experiment and other semiarid soils near by have been used in these investigations and have made it possible to compare the data obtained in the laboratory studies with the effects produced in the field.

The relative rates of nitrification in the field and laboratory, the effects of soil treatments including different fertilizers, manure, and cover

crops, the effects of alkali salts, the relative nitrifiability of different fertilizing substances, seasonable variation in nitrification, the movement of nitrates, and other phases of this question are being studied. The results obtained in the early stages of this work strongly emphasized the need for further study of the methods to be used.

In the previous studies on nitrification in semiarid soil the Remy-solution method, with certain modifications, has been used to a limited extent; but usually the direct-soil method has been used, in which actual soil is employed as the culture medium.[1] Different investigators, however, have modified the details to suit their own ideas. These modifications have to do mainly with variations in temperature, moisture content, and periods of incubation on the one hand, and differences in the ratio of soil to nitrogenous materials on the other. Regarding the latter it is noteworthy that the percentage of nitrogenous, organic material employed has been varied from about 0.7 to 2 per cent. In the case of ammonium sulphate the variations have ranged from 0.1 to 1 per cent.

Likewise, widely variant percentages of actual nitrogen from different sources have been added by one and the same investigator. For example, Lipman and Burgess (24) employed equal weights (1 per cent) of calcium cyanamid, dried blood, bone meal, high-grade tankage, cottonseed meal, manure, etc.; but since the nitrogen content of these materials ranged from 2.46 per cent to 16.55 per cent, the actual quantities of nitrogen added must have varied accordingly. Ammonium sulphate was added at the rate of 0.2 per cent. On the basis of the data thus obtained, deductions were made concerning the relative nitrifiability of these materials. Likewise, McBeth and Smith (27) added dried blood and ammonium sulphate at the rates of 1 and 0.08 per cent, respectively; and while the absolute amounts of nitrate formed from the latter were considerably larger than from the former, no particular notice was given to it, an average of the results from the two forms being recorded in many cases.

Before presenting the full data bearing upon the specific subjects named above, the present paper will be devoted to a discussion of the methods commonly employed in laboratory studies on nitrification with special reference to the concentration of nitrogenous materials and period of incubation used. In most cases the phenol-disulphonic-acid method was used for the determination of nitrate. At frequent intervals throughout this investigation the aluminum reduction method as outlined by Burgess (5) was also used for the purpose of checking the results obtained by the colorimetric method. The results by the two methods were found to agree closely in all cases, except where high concentrations of nitroge-

[1] It is not deemed necessary to discuss in detail the advantages and disadvantages of these two methods. The reader is referred to a paper by Löhnis and Green (25) in which a critical review of the subject is given. Some of the points emphasized below were also strongly emphasized by them two years previously.

nous materials or other abnormal conditions were employed. In such cases the results by the reduction method were frequently much higher than by the colorimetric method. Further reference will be made to this point later. It is recognized that the colorimetric method is not accurate where high concentrations of nitrate occur, but the results are believed to be sufficiently accurate for the purposes of this paper.

EXPERIMENTAL WORK

The soil used was for the most part drawn from the fertilizer plots[1] referred to above. This soil has been derived from the disintegration of monzonite and is a light, sandy loam, very low in organic matter and nitrogen. It is underlain with a deep subsoil similar in nature to the surface soil. A composite sample composed of about 20 borings was obtained from each plot sampled and also from the virgin soil[2] near by. The samples were taken to a depth of 6 inches with a King soil tube and were then immediately brought to the laboratory and spread out on clean paper to dry. After becoming air-dry and being thoroughly mixed, duplicate portions of 150 gm. were mixed in tumblers with 1.5 gm. of dried blood, and the moisture content made up to 15 per cent with distilled water, after which the samples were incubated at 25° to 28° C.

In order to ascertain the formation of ammonia, 50-gm. portions were withdrawn at the end of 7 and 28 days, and the ammonia determined by distilling with an excess of magnesium oxid, and at the end of 28 days the nitric nitrogen was determined. The average of closely agreeing duplicates is recorded in Table I.

TABLE I.—*Ammonification and nitrification of 1 per cent dried blood (in parts per million)*

Soil.	Ammonia nitrogen.		Nitric nitrogen.	
	After 7 days.	After 28 days.	Original soil.	Gain in 28 days.
Virgin	584	385	1.2	5.5
Control plot	503	400	2.1	2.5
Manured plot	497	287	8.4	241.6

The foregoing data show that active ammonification took place in the soil from each plot studied and that a relatively high concentration of ammonia still occurred at the end of 28 days. The lesser amount of ammonia found in each case at the end of 28 days was probably due in part to the loss of ammonia by volatilization. Strong odors of ammonia were detected, especially in the tumblers containing the virgin soil and

[1] It is not deemed necessary at this point to discuss in detail the different treatments that have been applied in the field experiments. A more complete discussion will be presented in a subsequent paper.

[2] The term "virgin soil" as used in this paper signifies uncultivated soil still bearing native vegetation.

that from the control plot. It is not deemed necessary to dwell further on the ammonification of organic materials in these soils. Suffice it to say that the ammonia has been determined in a large number of instances throughout this work and without exception ammonification has been found to be active in every soil studied. The above data on ammonification are submitted merely to show that nitrification was not limited by inactive ammonification and not as a special contribution to the study of ammonification.

Considering the amounts of nitric nitrogen found, it will be noted that little or no nitrification took place except in the soil previously treated with manure. In this case quite active nitrification took place. It is of special interest that only the most enfeebled nitrification of dried blood took place in the virgin soil; and although slightly more nitrate was formed in the virgin soil than in the check plot, the difference is too small to be noteworthy.

Two of the plots (C and S) in the field experiments from which the above samples were drawn have been annually fertilized with dried blood for the past nine years (1907–1915). During the past two years (1914–15) the application has been made at the rate of 1,080 pounds per acre. Notable stimulation in the growth and yield of fruit has been produced. One of these plots (C) lies adjacent to the check plot used in the foregoing experiments. Soil samples drawn at frequent intervals during the past two years from this and other plots fertilized with dried blood have consistently shown a well-defined increase in nitric nitrogen over that in the unfertilized plots. Furthermore, considerable increases in the nitrate content have been found following each application of dried blood. It would seem, therefore, that dried blood undergoes active nitrification in the field where no other form of organic matter has been applied, notwithstanding the fact that the above data indicate that both the virgin soil and control plot are unable to nitrify dried blood.

Two questions presented themselves: First, why does dried blood undergo active nitrification in the field but not in the laboratory? Second, why is it that dried blood undergoes active nitrification in the soil from the manured plot but apparently not in the control plot? Considerations arising out of these questions have led to an extended study of the factors affecting nitrification in the field and laboratory.

NITRIFICATION AS AFFECTED BY VARYING CONCENTRATIONS OF MATERIALS

As stated above, 1,080 pounds of dried blood per acre have been applied annually for the past two years (1914–15) to plots C and S. Until the present year (1916) only one-third of this quantity was applied at one time, the remaining two-thirds being applied at intervals of about two months each. But assuming that the entire amount becomes thoroughly mixed with the soil to a depth of 6 inches and estimating that the

soil weighs 2,000,000 pounds per acre of 6 inches, the average concentration of dried blood that obtains in the field would be 0.054 per cent. On the other hand, a concentration of 1 per cent was employed in the preceding laboratory experiments, which is 18.5 times that which obtains in the field.

It was at once suggested that an excessive concentration had been used in the laboratory. Accordingly a preliminary set of experiments was made, in which widely different concentrations of dried blood were added. The result was that the soil from the plots which had previously failed to nitrify a 1 per cent concentration of dried blood was found to support active nitrification of this material when added in low concentrations.

An extended study has been made on the rates of nitrification of different nitrogenous materials when used in varying concentrations. Fresh samples were drawn from the same plots as in the preceding series. The nitrogeneous materials used were dried blood, bone meal, and ammonium sulphate, representing a high-grade and a low-grade organic form and an inorganic compound, respectively. The dried blood (13.20 per cent of nitrogen) was added in quantities ranging from 1 to 0.0625 per cent. The bone meal (4.25 per cent of nitrogen) was varied from 4 to 0.25 per cent. Ammonium sulphate (21 per cent of nitrogen) was varied from 0.6 to 0.0375 per cent. The experiments were made in duplicate. Control portions of each soil without the addition of nitrogenous material were also incubated. The incubation period was four weeks. The results are given in Table II.

TABLE II.—*Nitrification as affected by different concentrations of nitrogenous materials*

Materials added.	Nitrogen added per 100 gm. of soil.	Virgin soil.		Control plot.		Manured plot.	
		Nitric nitrogen found.	Percentage nitrified.	Nitric nitrogen found.	Percentage nitrified.	Nitric nitrogen found.	Percentage nitrified.
	Mgm.	*P. p. m.*		*P. p. m.*		*P. p. m.*	
None	0	34.0	.....	19.6	.....	38.7	.....
Dried blood:							
1.0 per cent	132.0	22.0	0	3.8	0	316.0	21.0
0.5 per cent	66.0	42.0	1.2	21.0	0.2	248.0	31.7
0.25 per cent	33.0	57.5	7.1	120.0	30.4	140.0	30.7
0.125 per cent	16.5	111.0	46.6	99.0	48.1	96.0	34.8
0.0625 per cent	8.25	73.0	47.3	75.0	67.1	82.0	52.5
Bone meal:							
4.0 per cent	170.0	24.5	0	18.9	0	188.0	8.8
2.0 per cent	85.0	51.5	2.1	27.5	0.9	240.0	23.7
1.0 per cent	42.5	109.0	17.7	96.0	17.9	220.0	42.7
0.5 per cent	21.25	99.5	30.9	104.0	39.7	150.0	52.4
0.25 per cent	10.62	80.0	43.3	69.0	46.5	91.0	49.3
Ammonium sulphate:							
0.6 per cent	126.0	15.4	0	31.0	0.9	94.0	4.4
0.3 per cent	63.0	53.0	3.0	63.0	6.9	176.0	21.8
0.15 per cent	31.5	65.0	9.8	82.0	19.8	158.0	37.9
0.075 per cent	15.7	78.0	28.0	88.0	43.6	148.0	69.6
0.0375 per cent	7.85	69.0	44.6	74.0	69.3	112.0	93.4
Original soil		1.8	.....	4.5	.....	13.6	.....

As shown above, 1 per cent of dried blood failed to be nitrified in the virgin soil and that from the control plot, but underwent active nitrification in the previously manured soil as in the preceding series. The larger quantities of bone meal and ammonium sulphate also failed to be nitrified, but the lower concentrations of each of these substances underwent active nitrification in every case. Dried blood in a concentration of 0.625 per cent, which corresponds closely with that used in the field, underwent active nitrification in all cases. The percentages of nitrification, calculated after subtracting the amounts of nitric nitrogen found in the control portions, show that when corresponding amounts of actual nitrogen from the different sources are compared the rates of nitrification of dried blood, bone meal, and ammonium sulphate were quite similar in all cases with the single exception of ammonium sulphate in the manured soil. In this case ammonium sulphate was oxidized the most completely of any of the materials studied.[1]

Soils from other localities have also been studied. Two samples were obtained from the lemon groves of a ranch in Ventura County. One of these (A) is a light sandy soil; the other (B), a heavy adobe soil high in organic matter. A sample was taken from a young lemon grove on another ranch in Ventura County and is a heavy clay soil, containing considerable organic matter. Another sample of a light sandy character was taken from an orange grove opposite the Lark Ellen station near Covina. A sandy loam containing considerable gravel and organic matter was obtained from a 24-year-old orange grove in the La Verne section in southern California.

Studies were made in duplicate with the use of the same materials as in the preceding series. Since a 2 per cent concentration of dried blood (8, 13, 18) has been previously used to some extent in studies on nitrification, this proportion was added in certain cases. The concentrations of bone meal were varied from 4 to 0.5 per cent, and of ammonium sulphate, from 0.3 to 0.075 per cent. The results are given in Table III.

TABLE III.—*Nitrification in soils from different localities*

Materials added.	Nitrogen added per 100 gm. of soil.	Sespe soil (A).		Sespe soil (B).		Limoneira soil.		Lark Ellen soil.		La Verne soil.	
		Nitric nitrogen found.	Percentage nitrified.	Nitric nitrogen found.	Percentage nitrified.	Nitric nitrogen found.	Percentage nitrified.	Nitric nitrogen found.	Percentage nitrified.	Nitric nitrogen found.	Percentage nitrified.
	Mgm.	*P.p.m.*		*P.p.m.*		*P.p.m.*		*P.p.m.*		*P.p.m.*	
None	0	61.0	.....	38.5	.....	49.0	.....	86.0	.....	34.0	.....
Dried blood:											
2.0 per cent	264.0	.....	.....	.....	.....	6.5	0	24.5	0	.....	.....
1.0 per cent	132.0	736.0	51.1	360.0	24.3	468.0	31.7	107.0	1.6	442.5	30.9
0.125 per cent	16.5	170.0	66.1	129.0	54.9	163.0	69.1	191.0	63.6	144.0	66.6
Bone meal:											
4.0 per cent	170.0	220.0	9.4	224.0	10.9	332.0	16.6	76.0	0	.....	.....
1.0 per cent	42.5	.....	.....	.....	.....	196.0	34.6	222.0	32.0	.....	.....
0.5 per cent	21.25	186.0	58.8	136.0	45.9	152.0	48.5	168.0	38.6	140.0	49.8
Ammonium sulphate:											
0.3 per cent	63.0	228.0	26.5	111.5	11.6	128.0	12.5	162.0	12.1	.....	.....
0.75 per cent	15.75	206.0	92.1	106.0	42.9	148.0	62.9	172.0	54.6	124.0	57.1
Original soil	.....	37.0	.....	25.5	.....	31.5	.....	45.5	.....	23.0	.....

[1] It should be stated that the addition of calcium carbonate exerts almost no effect on the nitrification of dried blood in this soil. A preliminary report on the above phases of this investigation has previously been issued (15).

Again, it was found that the lower concentrations of these materials were actively nitrified in every case; and when the concentration was increased, the percentage of nitrification decreased. One per cent dried blood was actively nitrified in every soil except that from Lark Ellen, but 2 per cent was toxic in each case. The percentage of dried blood nitrified was somewhat greater than that of bone meal; otherwise the degrees of nitrification of the different materials were similar. The results, therefore, are in harmony with those of the preceding series.

The preceding data show that each of the soils studied, representing quite a wide range of soil conditions, is capable of supporting active nitrification of dried blood, bone meal, or ammonium sulphate, provided these materials be added in low concentrations. They also indicate that the results obtained with the use of such high concentration of dried blood as 1 and 2 per cent, or 0.3 and 0.6 per cent of ammonium sulphate, do not form a reliable criterion upon which to base practical conclusions.

NITRIFICATION AT DIFFERENT DEPTHS AS AFFECTED BY CONCENTRATION

As already stated, differences of opinion are held regarding nitrification in the substrata of semiarid soils. The following data are of interest in this connection. The soil used was taken from an orange grove near Woodlake, in Tulare County. It is a dark-colored clay loam, high in organic matter. The subsoil contains considerably less organic matter than the surface soil and closely resembles adobe. The samples were drawn in foot sections down to 5 feet in depth. Only a few concentrations of nitrogenous materials were employed, owing to the smallness of the samples. The incubation period was four weeks, as in the previous series. (Table IV).

TABLE IV.—*Nitrification in soil from different depths*

Materials added.	Nitrogen added per 100 gm. of soil.	First foot.		Second foot.		Third foot.		Fourth foot.		Fifth foot.	
		Nitric nitrogen found.	Percentage nitrified.	Nitric nitrogen found.	Percentage nitrified.	Nitric nitrogen found.	Percentage nitrified.	Nitric nitrogen found.	Percentage nitrified.	Nitric nitrogen found.	Percentage nitrified.
	Mgm.	*P.p.m.*		*P.p.m.*		*P.p.m.*		*P.p.m.*		*P.p.m.*	
None	0	60.0	.....	64.0	.....	46.0	.....	36.0	.....	35.0	.....
Dried blood, 1 per cent	132.0	32.0	0	2.6	0	1.8	0	0.1	0	9.2	0
Dried blood, 0.1 per cent	13.2	132.0	54.5	95.0	23.5	100.0	40.9	40.0	3.0	34.0	0
Ammonium sulphate, 0.0625 per cent	13.2	130.0	53.0	92.0	21.2	74.0	21.2	68.0	24.2	68.0	25.0
Original soil	.....	1.1	.....	4.5	.....	2.5	.....	3.6	.....	0.6	.....

Active nitrate formation took place in the check portions from each of the 5-foot sections studied, showing that the nitrifying organisms are not only present down to 5 feet in depth but that the chemical, physical, and biological conditions ensuing in the samples were favorable for nitri-

fication. The results show, furthermore, that 1 per cent of dried blood may be an excessive concentration, even in a highly organic soil; less nitrate was formed in the samples for each foot where 1 per cent of dried blood had been added than in the control portions.

A concentration of 0.1 per cent of dried blood, or an equivalent amount of nitrogen in the form of ammonium sulphate, underwent vigorous and practically equal nitrification in the soil from the first foot and was also actively nitrified in the subsoils from the second and third feet. Ammonium sulphate likewise underwent considerable nitrification in the samples from the fourth and fifth feet; but the amounts of nitrate produced with a 0.1 per cent concentration of dried blood in soil from these depths were approximately the same as in the checks, indicating that a concentration of 0.1 per cent of dried blood may be excessive.

Considering the fact that nitrification took place in the check portions and where ammonium sulphate was added, the conclusion seems warranted that the subsoil from this orchard at least possesses the potential capacity of producing nitrates down to a depth of 5 feet.

However, the writer does not consider it safe to conclude from the preceding data that active nitrification takes place in the field in the subsoils of the orchard from which the above sample was drawn, since much more thorough aeration took place after the samples were drawn than ordinarily takes place in the subsoil *in situ*.

The results, as a whole, again emphasize the importance of employing low concentrations of nitrogenous materials and show that the inability to nitrify a concentration of 1 per cent of dried blood is not confined to humus-poor soil, as suggested by Lipman and Burgess (24).

EFFECTS OF ALKALI SALTS ON NITRIFICATION AS MODIFIED BY THE CONCENTRATION OF NITROGENOUS MATERIALS

One of the most important soil questions in the semiarid region relates to the effects of alkali salts, particularly the carbonate, chlorid, and sulphate of sodium. As already stated, Lipman and his coworkers (17, 18, 19, 22) have devoted considerable study to the biochemical effects of these salts. But the conclusions which were drawn relative to nitrification were based on the effects produced with a concentration of 1 or 2 per cent of dried blood. In the light of the results presented above, it becomes a matter of interest to study the effects of alkali salts with the use of varying concentrations of nitrogenous materials.

The soils used were drawn from the check and manured plots; the required amounts of the salts were added in solution after the dried blood or ammonium sulphate had previously been mixed with the soil. The same percentage of moisture and an incubation period of four weeks were employed, as in the previous series. Table V gives the results of this series.

TABLE V.—*Effect of alkali salts on the nitrification of dried blood and ammonium sulphate*

Alkali salt added.	Nitric nitrogen (per million).					
	Control plot.			Manured plot.		
	0.1 per cent of dried blood.	0.0625 per cent of ammonium sulphate.	0.15 per cent of ammonium sulphate.	1.0 per cent of dried blood.	0.1 per cent of dried blood.	0.15 per cent of ammonium sulphate.
None	108.0	98.0	89.0	172.0	106.0	170.0
Sodium carbonate:						
0.05 per cent	.....	.....	56.0	31.0	108.0	129.0
0.1 per cent	104.0	160.0	33.5	19.0	102.0	62.0
0.5 per cent	1.5	1.9	1.1	7.6	[a] 110.0	5.6
Sodium sulphate:						
0.1 per cent	115.0	96.0	60.0	296.0	103.0	174.0
0.5 per cent	98.0	95.0	41.0	48.0	102.0	134.0

[a] 0.4 per cent of sodium carbonate was used in this case.

Considering the results from the control plot, it is interesting to note that 0.1 per cent of sodium carbonate produced no effect upon the nitrification of a concentration of 0.1 per cent of dried blood, was distinctly toxic to the nitrification of a concentration of 0.15 per cent of ammonium sulphate, and markedly stimulating to the nitrification of a concentration of 0.0625 per cent of ammonium sulphate. A concentration of 0.5 per cent of sodium carbonate was toxic in all cases.[1]

With a 1 per cent concentration of dried blood in the soil from the manured plot, the addition of a concentration of 0.05 per cent of sodium carbonate was quite toxic to nitrification, causing a reduction in the yield of the nitrate from 172 to 31 p. p. m. A 0.1 per cent concentration of sodium carbonate was still more toxic, while a concentration of 0.5 per cent totally inhibited nitrification. (The original soil contained 6.7 p. p. m.) With the use of 0.1 per cent dried blood no effects were produced by any of the concentrations of sodium carbonate employed. With the use of a concentration of 0.15 per cent of ammonium sulphate the addition of 0.05 or 0.1 per cent sodium carbonate retarded nitrification considerably, especially in the case of the latter, but not so markedly as was found in the case of 1 per cent dried blood.

The addition of a concentration of 0.1 per cent of sodium sulphate was without effect on the nitrification of 0.1 per cent dried blood or an equivalent amount of ammonium sulphate in the control plot, and upon the nitrification of concentrations of 0.1 per cent of dried blood or 0.15 per cent of ammonium sulphate in the manured plot; but it produced marked stimulation with 1 per cent dried blood in the manured plot and was toxic with 0.15 per cent ammonium sulphate in the control plot. A concentration of 0.5 per cent of sodium sulphate was somewhat toxic with 1

[1] The chemistry of the action of sodium carbonate and other sodium salts on this soil will be discussed in a subsequent paper. Suffice it to say that considerable light has been thrown on the above results from a study of the pure chemistry involved.

per cent dried blood and 0.15 per cent ammonium sulphate; but it produced no effects on the nitrification of a concentration of 0.1 per cent of dried blood.

With one exception it is noteworthy that the effects produced by either sodium carbonate or sodium sulphate were quite similar when equal amounts of nitrogen in the form of dried blood and ammonium sulphate were employed in low concentrations.

The above results are in harmony with those of Lipman (18) in showing that sodium carbonate is extremely toxic to the nitrification of a high concentration of dried blood and far more toxic than sodium sulphate.

NITRIFICATION DURING DIFFERENT LENGTHS OF TIME

In the preceding studies the samples were incubated for four weeks, and, as already stated, conclusions on the relative rates of nitrification of different materials and in different soils have frequently been drawn from data obtained in this way. In the light of the preceding results it becomes a matter of interest to study nitrate formation at different intervals of time. In a preliminary study with the use of 1 per cent of dried blood in soil from the control plot it was found that no nitrification took place during a period of 68 days. In another series with the use of the same soil the incubation period was extended to 105 days, with the same result. With still other soils in which a concentration of 1 per cent of dried blood failed to be nitrified in four weeks, it has been found, however, that active nitrification may set in later, and in some cases eventually becomes quite as active as in soils which have the power of nitrifying 1 per cent vigorously within four weeks.

For the purpose of studying nitrification progressively with low concentrations of materials, 2,000 gm. of the fresh soil from the check and manured plots were kept in large jars. Dried blood and ammonium sulphate were added in quantities supplying 10 mgm. of actual nitrogen per 100 gm. of soil. The mositure content was brought up to 15 per cent and maintained near this point by the occasional addition of distilled water as evaporation took place. The soils were incubated as before. One-hundred-gm. portions were withdrawn at intervals and the nitrate determined, as shown in Table VI.

TABLE VI.—*Nitrification during different intervals of time*

Soil.	Nitrogenous materials added.	Nitric nitrogen (parts per million).						
		Original soil.	After 4 days.	After 7 days.	After 9 days.	After 15 days.	After 31 days.	After 94 days.
Control plot...	None.....	1.2	4.0	7.0	7.4	9.0	7.5	16.5
Manured plot..	.....do.....	4.9	9.8	10.0	12.0	13.2	14.0	23.0
Control plot...	Dried blood.....	1.2	2.2	10.4	33.5	66.0	60.0	72.0
Manured plot..	.....do.....	4.9	10.4	44.0	58.0	73.0	64.0	64.0
Control plot...	Ammonium sulphate.	1.2	4.4	16.5	37.0	65.0	76.0	88.0
Manured plot..	.....do.....	4.9	14.4	60.0	72.0	96.0	96.0	96.0

The above data show that nitrification set in within the first four days and continued in the untreated portions throughout the 94 days of the experiment. When allowance is made for the nitrate originally present, it will be seen that almost as active nitrification took place in the untreated soil from the control plot as in that from the manured plot. But with the addition of 10 mgm. of nitrogen in the form of dried blood, the more active nitrification took place during the first nine days in the soil from the manured plot. After this time nitrate formation took place the more vigorously in the soil from the control plot.

Ammonium sulphate was most actively nitrified in the manured soil during the first 31 days. After this time the rate in the control plot exceeded that in the manured plot. At every time interval, with the exception of the 15-day period, ammonium sulphate was found to undergo more active nitrification than dried blood.

If the rates of nitrification in the two soils be compared on the basis of the data obtained upon the ninth day,[1] it would seem reasonable to conclude that the manured plot is capable of supporting more active nitrification of either dried blood or ammonium sulphate than the check plot. If a later period be chosen the inference seems equally reasonable that the two soils are about equal in ability to nitrify dried blood. The data obtained from the untreated portions, however, would seem to indicate that the floras of the two soils are quite similar, so far as their ability to produce nitrate is concerned. In a subsequent paper this point will be more fully discussed.

The preceding data strongly emphasize the importance of studying the formation of nitrates in laboratory studies during different intervals of time and in the presence of varying concentrations of different nitrogenous materials. Just as different concentrations of nitrogenous materials, as already stated, may lead to widely different conclusions, the above results show that almost any conclusion may be drawn regarding the relative rates of nitrification of dried blood and ammonium sulphate in a given soil or of dried blood in different soils, provided the incubation periods be carefully chosen.

ACCUMULATION OF NITRITES IN LABORATORY EXPERIMENTS ON NITRIFICATION

From the classical experiments of Winogradsky it is generally considered that nitrification proper begins with ammonia and takes place in two stages, each stage being brought about by a different set of bacteria. The nitrite bacteria oxidize the ammonia to nitrous acid, and the nitrate bacteria complete the oxidation to nitric acid. In field soils, however, the activity of the latter is usually sufficiently great to complete the oxidation of nitrite almost, if not quite, as fast as it is formed. Hence, nitrite rarely accumulates in notable amounts in arable soils.

[1] Such comparisons have previously been made (8, 27) upon the basis of a 10-day incubation period.

However, nitrite may accumulate to a considerable extent under poorly aerated conditions, especially when artificial applications of nitrate fertilizers are made, but under such conditions it is highly probable that the nitrite is formed in part at least through the reduction of nitrates rather than from the incomplete oxidation of ammonia. The writer (12) has shown, for example, that the application of sodium nitrate to rice soils immediately preceding or during the time of submergence may result in an accumulation of considerable amounts of nitrite. The addition of large amounts of carbohydrates may also bring about a similar reduction of nitrates, even under aerobic conditions. In general, it may be said that the accumulation of notable amounts of nitrite in soils is an indication of the existence of unfavorable soil conditions.

Notable amounts of nitrite have previously been found in laboratory incubation experiments on nitrification. In experiments with the use of asparagin Withers (35) found considerable amounts of nitrite in certain soils in North Carolina, while only slight nitrate formation took place. On the other hand, ammonium sulphate was oxidized to nitrate without the accumulation of more than a trace of nitrite. In sterilized portions of this soil, which were later exposed to reinoculation, notable amounts of nitrite were formed within four weeks' time from both asparagin and ammonium sulphate, but practically no nitrate was formed from either. The amounts of nitrogenous materials used in these experiments were not stated.

Sackett (28) also found considerable amounts of nitrite in laboratory experiments. He used 100 mgm. of actual nitrogen in the form of ammonium sulphate, ammonium chlorid, ammonium carbonate, and dried blood per 100 gm. of soil, which corresponds, in the case of ammonium sulphate, to a concentration of about 0.5 per cent, and in that of dried blood approximately to 0.75 per cent. The incubation period was six weeks. It is notable that in certain soils he found that nitrite formation took place much more rapidly than nitrate formation, and in other soils there was evidence of nitrite formation through the reduction of nitrate. In the control portions to which no nitrogenous materials were added the concentrations of nitrite did not amount to more than 1 to 2 p. p. m. It is probable that the results obtained by Sackett would have been greatly different had he employed a lower concentration of the nitrogenous materials.

The writer (13) has also previously found considerable amounts of nitrite in laboratory experiments with the use of excessive amounts of magnesium carbonate. In these experiments a concentration of 2 per cent of dried blood was employed with the light, sandy soil from Anaheim, Cal. In discussing this point it was tentatively suggested that the nitrite found arose from the reduction of nitrate, and that the magnesium carbonate was more toxic to the nitrifying bacteria than to the denitrifiers. In the light of evidence obtained more recently it seems

more probable, however, that the magnesium carbonate in low concentrations was toxic to the nitrate formers but not to the nitrite formers, whereas in still higher concentrations it was toxic to both groups.

In the course of some studies on the effects of concentration on the nitrification of ammonium carbonate it was observed that notable amounts of nitrite began to accumulate as the concentration was increased above 15 mgm. of nitrogen per 100 gm. of soil; with a concentration of 30 mgm. the nitrite content, after four weeks' incubation, was found to be 268.5 p. p. m., while at the same time no nitrate was formed. From these observations it would seem that the nitrate bacteria are more sensitive to high concentrations of ammonium carbonate than the nitrite group, as has been definitely shown to be the case by Boullanger and Massol (4).

The effects on nitrite accumulation brought about by the concentration of different nitrogenous substances and in different soils, the effects produced by the addition of alkali salts, organic matter, etc., have been studied at varying intervals of time. The full data will be presented in a later paper. Briefly, it may be stated that not more than a few tenths p. p. m. of nitrite have been found where low concentrations of nitrogenous materials have been used, but that as the conditions become increasingly abnormal, either through the use of excessive amounts of nitrogenous substances, the addition of alkali salts, or by other means, a point is usually reached where nitrite formation proceeds more vigorously than nitrate formation, with a consequent accumulation of considerable amounts of nitrite. In addition, it has been found that under certain conditions nitrite formation may proceed vigorously without nitrate formation taking place at all, even in a soil where the nitrate bacteria are present in abundance.

The occurrence of notable amounts of nitrite necessitates some departure from the methods usually employed in the determination of nitrate in soils. As already stated, the results obtained by the phenol-disulphonic-acid method were found to agree closely with those obtained by the aluminum-reduction method except where high concentrations of nitrogenous materials had been employed. In such cases the reduction method frequently gave much higher results.[1] As shown below, the use of the reduction method effects a conversion of nitrite into ammonia, just as is the case with nitrates; and consequently the results found represent the total of the nitrite and nitrate nitrogen present, although it is recorded as nitrate.

If a solution containing nitrite is evaporated and the residue then treated with the phenol-disulphonic-acid reagent, small amounts of nitrite may also be converted into nitrate, thus introducing a slight error.

After considerable experimentation the method adopted for the determination of nitrate in the presence of nitrite was as follows: The water

[1] As shown by Allen (1), certain soluble organic forms of nitrogen also become reduced to ammonia under the conditions employed in this method.

solution of the soil was made up as usual. To an aliquot portion, 10 c. c. of a 1 per cent solution of ammonium sulphate was added, then evaporated on the water bath, and the determination completed as usual with the phenol-disulphonic-acid method. During the evaporation the ammonium sulphate brings about complete decomposition of the nitrite through the formation of ammonium nitrite, which decomposes at the temperature employed.[1] The nitrite was determined by the Griess-Ilosvay method.

The following data (Table VII) will show the wide range of results obtained by the use of different methods:

TABLE VII.—*Effects of nitrite on the determination of nitrate (in parts per million)*

	Nitrite nitrogen.	Nitrate nitrogen by modified colorometric method.	Nitrate nitrogen by the usual colorometric method.	Nitrate nitrogen by aluminum reduction method.
Soil plus 1 per cent of dried blood incubated for 46 days	275	22	30	283

The above data show that nitrite becomes reduced to ammonia under the conditions employed in the aluminum reduction method. It is reasonable to infer, therefore, that unless allowance be made for the nitrite present the results obtained by the reduction method from incubations with high concentrations of nitrogenous materials will represent the total nitrite and nitrate present rather than the nitrate only (22).

It has been found that with the use of 1 per cent of dried blood nitrites may accumulate in large amounts in soils of various types and that the nitrite may persist without undergoing further oxidation, at least for a period of 105 days, as shown in Table VIII.

TABLE VIII.—*Accumulation of nitrite as affected by concentration (in parts per million)*

Soil.	Original soil.		After 28 days.		After 42 days.		After 56 days.		After 71 days.		After 105 days.	
	Nitrite nitrogen.	Nitrate nitrogen.	Nitrite nitrogen.	Nitrate nitrogen.	Nitrite nitrogen.	Nitrate nitrogen.	Nitrite nitrogen.	Nitrate nitrogen.	Nitrite nitrogen.	Nitrate nitrogen.	Nitrite nitrogen.	Nitrate nitrogen.
Control plot untreated	0	1.4	0	12.0	0	11.2	0	12	0	15	0	18
Control plot plus 0.0625 per cent of dried blood	0	1.4	Trace.	60.0	0	70.0	0	70	0	67	0	68
Control plot plus 1 per cent of dried blood	0	1.4	20	19.5	87.5	26.0	150	19	150	24	265	29
Manured plot untreated	0	3.2	0	14.0	0	17.0	0	17	0	26	0	25
Manured plot plus 0.0625 per cent of dried blood	0	3.2	Trace.	50.0	0	60.0	0	72	0	74	0	70
Manured plot plus 1 per cent of dried blood	0	3.2	107	160.0	56.0	272.0	75	300	0	465	0	416

[1] This procedure was suggested by the method formerly employed for the determination of nitrate in the presence of nitrite by Frankland (6).

The above shows that large amounts of nitrite may accumulate when high concentration of dried blood have been used, but with a low concentration such is not the case. It is noteworthy that high concentration of other nitrogenous materials, such as bone meal, ammonium sulphate, and ammonium carbonate, all have been found to promote the accumulation of large amounts of nitrite in incubation studies.

Alkali salts also exert marked effects upon the accumulation of nitrites as shown by Table IX.

TABLE IX.—*Effects of sodium carbonate on the accumulation of nitrite*

Nitrogenous material added.	Sodium carbonate added.	After two weeks.		After four weeks.	
		Nitrite nitrogen.	Nitrate nitrogen.	Nitrite nitrogen.	Nitrate nitrogen.
	Per cent.	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*
None	None.	0	16.2	0	48.0
0.1 per cent of dried blood	...do....	0	84.0	0	110.0
Do	0.1	Trace.	86.0	0	100.0
Do	0.25	25.0	19.2	25.0	102.0
Do	0.5	Trace.	1.5	0	2.0
0.0625 per cent of ammonium sulphate	None.	0	68.0	0	104.0
Do	0.1	Trace.	106.0	0	160.0
Do	0.25	62.5	10.0	93.8	79.0
Do	0.5	1.7	1.7	0.5	1.9

Not more than a mere trace of nitrite was found where a concentration of 0.1 per cent of sodium carbonate had been added, and no effect was produced on nitrate formation; but considerably greater amounts of nitrite than nitrate occurred after two weeks' incubation where a concentration of 0.25 per cent of sodium carbonate had been added. However, after four weeks most of the nitrite formed from dried blood had become oxidized to nitrate; while in the case of ammonium sulphate, the concentration of nitrite after four weeks still exceeded that of nitrate. The addition of 0.5 per cent of sodium carbonate entirely inhibited the formation of either nitrite or nitrate.

It has been found that a concentration of from 30 to 40 mgm. of nitrogen in the form of sodium nitrite per 100 gm. of soil entirely inhibits the formation of nitrate in the soil from the experimental plots. With lower concentrations the nitrite was completely oxidized in four weeks' time. These results taken in connection with the preceding indicate that when abnormal soil conditions are brought about, the concentration of nitrite produced in the oxidation of nitrogenous materials may become so high as in itself to inhibit nitrate formation.

CONCLUSIONS

In the preceding investigations it has been shown that the amounts of nitrate formed from dried blood, bone meal, or ammonium sulphate during four weeks' incubation varied enormously when different concentrations were employed. This is true in regard both to the absolute amount

of nitrate formed and the percentage of the nitrogen added that was nitrified.

When 1 per cent of dried blood was used, the nitrifying activity was found to be feeble or even negative in certain soils in which 1 per cent of bone meal and 0.2 to 0.3 per cent of ammonium sulphate underwent active nitrification, as was previously found by Lipman and Burgess (24). However, when low concentrations of dried blood were employed, such as are used in the field, active nitrification took place in every case; and when equal amounts of actual nitrogen were added, it was found that the yields of nitrate were quite similar, whether the nitrate had been derived from dried blood, bone meal, or ammonium sulphate. High concentrations of bone meal with a nitrogen content corresponding to that furnished by 1 per cent of dried blood were also toxic to nitrification, very much as was the case with 1 per cent of dried blood.

Experiments were made with widely different types of soil from a number of localities in southern California. It was found that the inability to nitrify 1 per cent of dried blood is not confined to any one type of soil nor to soils low in organic matter. The results as a whole seem to warrant the conclusion, however, that the soils of southern California in general are capable of supporting active nitrification of dried blood, provided it be added in concentrations corresponding to field practice.

It was found that the effects produced by the addition of alkali salts varied greatly when different concentrations of nitrogenous materials were employed. In a given soil a concentration of 0.05 per cent of sodium carbonate was distinctly toxic to the nitrification of 1 per cent of dried blood, while as high a concentration as 0.4 per cent produced no effects on the nitrification of 0.1 per cent of dried blood. Likewise, 0.1 per cent of sodium carbonate was toxic to the nitrification of 0.15 per cent of ammonium sulphate, and markedly stimulating when 0.0625 per cent of ammonium sulphate was used. Similar statements may be made with regard to the effects of sodium sulphate.

The results also show that widely different conclusions may be drawn from laboratory experiments when different periods of incubation are used.

Nitrites were found to accumulate in large amounts where excessive amounts of nitrogenous materials were employed. In some cases the nitrite content greatly exceeded the nitrate content after an incubation period of several weeks. Likewise, the addition of alkali salts may suppress nitrate formation, while at the same time permitting nitrite formation to proceed actively.

It is necessary to make allowance for the nitrite present in the determination of nitrate by the aluminum reduction or phenol-disulphonic-acid methods, but the error introduced by nitrite is far greater with the former method than with the latter.

It is not intended to give the impression from the above that all nitrogenous materials will undergo nitrification at equal rates when

present in low concentrations. It is recognized that different organic fertilizers undergo biochemical decomposition in varying degrees in a given soil, and this for a number of reasons, some of which will be discussed in a later paper. The writer holds, however, that the methods now employed by many students of nitrification, in which high concentrations of nitrogenous materials are added and the nitrate determined at a fixed interval of time, are not only unsatisfactory but that the results thus obtained are likely to be more misleading than informing.

In the light of the results obtained in this investigation, it seems highly probable that at least some of the peculiarities that have been noted in previous nitrification studies will be found to disappear under the more rational procedure of studying the activities of the organisms in an environment as nearly similar to that of the field as possible. The writer, while criticizing the methods in common use, freely admits that some of the conclusions previously drawn by him from studies with Hawaiian soils are open to serious question because of the methods employed (14).

The nitrate merely represents one of the end products formed; and in the case of an organic substance the intermediate products that are produced may, either directly or indirectly through the effect upon other organisms, exert much influence upon the oxidation of ammonia. In the presence of large amounts of materials it is highly probable that the relations of the different groups of organisms present become greatly changed, with a consequent effect on the oxidizing activity of the nitrifying organisms.

It is, of course, a matter of scientific interest that certain soils are capable of supporting active nitrification of 1 per cent of dried blood, while others are not, and the reasons underlying these differences are matters deserving further study, but so far as the practical side of nitrification is concerned the writer holds that laboratory experiments should be conducted under conditions as nearly comparable with those that obtain in the field as possible, and that at the present time nothing more than scientific interest can safely be attached to the results obtained with the use of such abnormally high concentrations of nitrogenous materials as are commonly used in laboratory experiments on this subject.

Many American bacteriologists have apparently accepted the conclusions of Stevens and Withers (29, 30) and have multiplied laboratory tests in a conventional way without seriously questioning the method. The result has been that the practical aspects of nitrification studies have become extremely empirical. While it has frequently been stated that nitrification in the laboratory is not strictly comparable with that in the field, the conditions obtaining in the nitrification of 1 per cent dried blood in the laboratory have been referred to as optimum conditions (27). The preceding data indicate, however, that such may be far from the case. The writer thoroughly agrees with the position taken by Löhnis and Green (25) and Allen and Bonazzi (2) in their recent discussions of this subject.

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FACTORS AFFECTING THE EVAPORATION OF MOISTURE FROM THE SOIL[1]

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INTRODUCTION

The importance of soil moisture in crop production is well understood. No plant can grow unless moisture is present to help make food available and furnish the water necessary to carry on regular plant functions. In arid regions the growth of crops is limited more by a lack of moisture than by any other factor, and even in regions of high rainfall crop yields are often materially reduced by droughts.

In sections where only a small amount of rain falls, practically all that sinks into the soil returns to the surface and is evaporated directly or passes through plants, from which it is evaporated. In humid regions also, where some of the soil moisture percolates to great depths, there is considerable loss by evaporation from the surface of the soil.

Moisture evaporated from the soil is completely lost and is of no value to crops; hence, it is important to reduce evaporation to a minimum, particularly where the supply of moisture is limited. The best condition would be to have no evaporation of moisture except that passing through the plant and assisting in its functions. Any information, therefore, that will lead to a better understanding of the factors involved in evaporation and a fuller knowledge of methods of controlling these factors will be of considerable practical importance as well as scientific interest.

Surface losses are due to two factors: (1) Capillarity, by which the moisture is brought to the surface, and (2) evaporation. In many of the soil-moisture studies that have been made these two factors have not been clearly separated, but have been considered together in determining loss. This has led to considerable confusion, since a difference in two losses might, in one case, be due to a difference in the rate at which moisture was supplied to the surface by capillarity and, in another case, to the evaporation factors.

In the experiments reported in this paper an attempt has been made to eliminate the factor of capillarity and to confine the studies entirely to evaporation in order to determine, as nearly as possible, the effect of a number of the factors involved in the evaporation of moisture from the soil.

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REVIEW OF THE LITERATURE

Considerable work has been done on various phases of the evaporation problem. One of the important factors influencing water losses is the wetness of the soil, or the initial percentage.

Widtsoe (19, p. 35, Table 20)[1] gives the results of four years' experimental work, as shown in Table I.

TABLE I.—*Total evaporation of water from bare College loam*

Moisture.	Loss in pounds per square foot.				
	1902	1903	1904	1905	Average.
Per cent.					
10	9	32	9	16	16
15	23	.....	10	30	21
20	51	80	38	69	60

Loss increased with the percentage of water up to 20 per cent, which was the highest degree of wetness used. He (19) says: "The evaporation of water from bare soils increased with the increased saturation of the soil. The increase in loss was usually much larger than the increase in saturation. In another treatise (20) he found that "the wetter the soil at the surface, the more rapid is the evaporation of water from it."

This is confirmed by Whitney and Cameron (18) and by Fortier (5). In Whitney and Cameron's work, 26 per cent was the highest humidity, that being used in but one set of experiments. Fortier's highest percentage of water was 17.5. In his conclusions he says, "The rate of evaporation from soils varies directly with the amount of moisture in the top layer."

The work of Cameron and Gallagher (3, p. 45-49) indicates that, after a certain wetness is reached, there is little if any increase in water loss. In their work the soils of different degrees of wetness were placed over sulphuric acid of different concentrations in order to control the humidity. Over 95 per cent sulphuric acid in desiccators, Podunk fine sandy loam gave the most rapid loss by evaporation up to 4 per cent moisture, with a very slight increase in loss up to 28 per cent. Miami black clay loam lost most rapidly up to 22 per cent, with a small increase to 41 per cent, where the loss was highest.

The color of the soil is claimed by King (10) to affect evaporation greatly, since the darker the soil the more heat it absorbs and radiates. He found that the rise in temperature, due to the darker color, is the important factor.

Concerning winds, King (9, p. 16) shows that up to 300 feet from woods the loss gradually increased with the distance. He (11) gives the following

[1] Reference is made by number to "Literature cited," p. 460–461.

data on evaporation for an hour from a wet soil with a surface of 27 square inches:

20 feet from hedgerow the evaporation was 10.3 c. c.
150 feet from hedgerow the evaporation was 12.5 c. c.
300 feet from hedgerow the evaporation was 13.4 c. c.

At 300 feet the evaporation was 30 per cent greater than at 20 feet and 6.7 per cent greater than at 150 feet, due largely to the vessels closer to the hedge having protection from the wind.

McDonald (12) gives this terse summary:

> Evaporation depends upon the temperature of the evaporating surface, the dryness of the air, and the velocity of the wind. The hotter the day, the greater the evaporation; the drier, the greater the evaporation—the ceaseless sucking up of moisture.

Bowie (1) claims that loss due to wind is caused by the more intimate contact of the air with the moist soil surface. He says:

> With average wind velocities of from 2.4 to 4.0 miles an hour, and with an average water temperature of 70 F., the increased evaporation rate due to wind was about 0.5 per cent a day for each mile of wind.

Payne (13), in giving the advantage of windbreaks in retarding evaporation, shows that a sod wall 4 feet high and 20 feet long, running east and west, reduces the loss from buckets placed in the ground level with the field. On the north side, buckets 1, 3, 5, 7, and 10 rods distant lost in 62 days moisture the equivalent of 677, 633, 700, 703, and 712 tons to the acre, respectively; on the south side, buckets 1, 3, 5, 7, and 8 rods distant gave losses of 647, 686, 738, 764, and 761 tons to the acre, respectively.

In work done by Principi (14) the conclusions reached were—

> That evaporation is most rapid from the materials which have the largest pore spaces, and that it remains almost the same whether it arises from a free water surface or from thin films covering the particles of wet material.

Woolny (21) claims that capillarity ceases when the diameter of the particles is more than 2 mm. and that it varies with smaller particles in proportion to their fineness—the finer the particles, the greater the lifting power, but the slower the movement.

In regard to moisture movement through a column of dry soil not in direct contact with the moisture but with a saturated atmosphere between, Buckingham (2, p. 9–18) says that moisture escapes probably by pure diffusion and that the loss in this way is proportional to the square of the porosity, following the same law as the diffusion of air and carbon-dioxid gas through soils.

Whitney and Cameron (18) showed that loams over a saturated atmosphere gave a greater diffusion of moisture through the pore spaces than through those of clay.

Fortier (5) shows, in the measurement of losses from water surfaces kept at different temperature, that reducing the temperature from 88° to 80.4° F. lessened evaporation 20 per cent; reducing it to 73.5° decreased evaporation 40 per cent; to 61.3° decreased it to 67 per cent; and to 53.4° reduced it to 85 per cent.

Shade, which is a great protection from heat, aids in preventing evaporation. Seelhorst (16) shows that a loam soil shaded by dry rye plants evaporated 13.9 per cent less of the rainfall than the same kind of soil unshaded.

Widtsoe's (20) work on the influence of shade on evaporation gives a loss of 22 pounds to the square foot as against 32 pounds for the unshaded portion; that is, there was 29 per cent greater loss in sunshine than in shade.

Fortier (6) shows that in actual experiments on Mount Whitney evaporation decreased with altitude. The decrease was rather regular, except at the summit (14,502 feet), where greater evaporation took place than at either 10,000 or 12,000 feet.

According to Carpenter (4), diminished barometric pressure tends to increase evaporation to the extent of 14 per cent at 9,000 feet and 18 per cent at 10,000 feet over that at 5,000 feet.

Mulching the surface of the soil a few inches by stirring it is the most common practice in use for the preservation of moisture under field conditions. Ridgaway (15) shows that stirring the surface to depths of 2, 4, and 6 inches in different tanks once a week, with the water level kept 22 inches below the surface of the soil, lessened evaporation by 19, 23, and 45 per cent, respectively, of the amount lost from unstirred soil. His work also shows that where water is maintained at 6, 12, 18, and 22 inches below the surface, the losses were 95, 70, 45, and 35 per cent of evaporation from a free-water surface. This bears out Wollny's (21) statement that "if there is water underneath the soil the evaporation decreases as the distance between the surface of this water and that of the soil increases."

Fortier (7) sums up the results when dry soil coverings were substituted for stirred surfaces, as shown in Table II.

TABLE II.—*Losses by evaporation from soil surfaces variously treated*

Locality.	Loss from unmulched soil.	Loss from 3-inch mulch on soil.	Loss from 6-inch mulch on soil.	Loss from 9-inch mulch on soil.	Number of days run.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	
Davis, Cal.	35.00	14.71	5.94	0.78	32
Wenatchee, Wash.	14.33	3.98	2.10	1.06	21
Reno, Nev.	20.39	8.26	2.74	1.96	21
Average.	23.24	8.98	3.59	1.27	24.7

Using enough water to cover the surface with 3.14 inches, this author (5) found that no mulch gave a 0.72-inch loss; a 4-inch mulch, 0.21 inch; an 8-inch mulch, 0.1 inch; and a 10-inch mulch, 0.03 inch in 14 days—that is, the various mulches saved in 14 days 16.24, 19.75, and 21.97 per cent of the amount applied.

The advantage of deep furrows as a saving in furrow irrigation is brought out by Fortier (5). When the same quantities of water were applied to tanks in furrows 3, 6, 9, and 12 inches deep, he found that the losses at the end of 10 days were 25, 18, 10.2, and 6 per cent of the total water applied. The loss was most rapid for the first 2 days, and at the end of 5 days 77 per cent of the total loss for 10 days had occurred.

Widtsoe's (19) work shows that in the two treatments, sand containing 15 per cent, sandy loam containing 20 per cent, and Sanpete clay containing 25 per cent moisture, the average loss from the soil receiving surface irrigation was more than three times as great in the same length of time as that from a subirrigated soil. Fortier (5) shows that soil subirrigated 2 feet underground by pipes lost only 25 per cent as much water in 20 days as when irrigated by surface flooding.

According to Stigell (17), bacterial growth retards evaporation. He says that this is attributed to utilization of moisture by the organisms in their metabolic products, and reduction of the porosity of the medium by the metabolic products of the organisms. Hoffman's (8) work shows that after the bacteria in various culture media were added to the soil, evaporation was increased except in the case of manure. In drawing his conclusions he leads one to believe that if the experiment is of long duration the results may be reversed, due to the accumulation of carbon dioxid from the organism being taken up by the soil moisture, thereby increasing the surface tension of the water. Gelatin was found to retard evaporation and for that reason could not be used as a culture media.

EXPERIMENTAL WORK

INITIAL QUANTITY OF SOIL MOISTURE

To find, if possible, the specific influence of varying percentages of water in soils, a series of experiments was conducted at the Utah Agricultural Experiment Station in the years 1912 to 1916, inclusive. The preliminary tests show that evaporation losses increased rapidly with the increased wetness of the soil, or with the initial quantity. The latter studies were so arranged as to locate as many points of variation in the losses as possible.

Preliminary Study

Late in June, 1913, a study of the effect of initial quantity of moisture in the soil was begun. One hundred gm. of dry Greenville loam were put in small, weighed tin plates about 5 inches in diameter, and puddled

with water. The soil was then set aside to dry. When the condition of dryness was approached, the moisture was made up to the desired content. Twelve percentages—5, 7½, 10, 12½, 15, 20, 25, 30, 35, 40, 45, and 50—were run in triplicate. The pans were set on a table in a large laboratory room. Weighings were taken daily to the nearest one-tenth of 1 gm. and water was added to make up the loss. This experiment continued for 42 days, at the end of which time the losses were computed for each week and for the entire period. Table III gives the losses for each period.

TABLE III.—*Effect of initial quantity of water on evaporation*

Soil moisture.	Water evaporated.	Soil moisture.	Water evaporated.
Per cent.	*Gm.*	*Per cent.*	*Gm.*
5	96.4	25	956.1
7½	229.2	30	1,013.3
10	363.0	35	1,048.1
12½	484.8	40	1,074.5
15	594.0	45	1,148.1
20	822.2	50	1,185.8

An examination of Table III shows that evaporation increased rapidly to about 20 per cent, less rapidly from 20 to 30 per cent, and slowly from 30 to 50 per cent.

LATER EXPERIMENTS

Various later studies were made with loam, sand, clay, and muck. The methods were similar to those used in the preliminary work, but the experiments were much more detailed and thorough. Most of the work was done in small tin plates and copper vessels 8 inches in diameter and 4 inches deep, though some tests were made in Petri dishes, some in long galvanized-iron tanks and some in deep cylindrical galvanized cans.

LOAM

The test with loam was conducted in the manner already described with 100 gm. of dry soil in tin plates. The percentages of moisture ran from hygroscopic water, which was about 1.8 per cent, to 50 per cent. The experiment was conducted for 13 days, from August 29 to September 12, the pans being made up to the original weight each day and shifted on the table to eliminate, in part, the influence of air currents.

A similar test was made during the winter in a steam-heated laboratory. All percentages from 1 to 40 were run in triplicate with 1 per cent intervals. Weighings were made each day and losses were considerably greater than those for the same number of days in the other trial. A possible explanation for this is that the laboratory under artificial heat

was slightly warmer and changed air much more frequently than in summer. The hygrometer showed somewhat lower humidity, but it could not show the influence of thorough ventilation.

The next study was made between June 4 and June 17, 1914. It was conducted in the same fashion, except that weighings were made twice daily, between 6 and 8 o'clock morning and evening. Four pans, instead of three, were used with each percentage from 1 to 50. The pans for 1 per cent gained up to about 1.8 per cent and then neither lost nor gained noticeably.

In addition to the pans containing wet soil, a pan of free water was exposed at each corner of the table on which the test was made. The two pans on the east and nearer the doors lost somewhat more than the two at the west end. All soils that approached saturation lost more than the free water. The losses from the 20 per cent pans were about equal to the average of those from the free-water pans.

An almost identical test was made in July which practically duplicated the former results. As 12 weighings were made, the length of this study was equivalent to 6 days.

These various experiments are combined in figure 1, which gives results for a total of 51 days with moisture up to 40 per cent, and 36 days with moisture up to 50 per cent. The curve for the 36-day results shows a rapid increase in evaporation with a higher initial percentage of moisture in the soil up to 7 or 8 per cent; then a less rapid increase up to 18 or 20 per cent, from which point the increase is small. In the tests continued for 51 days, the same general changes in the curve are noted, although the total loss is decidedly more than for the 36-day test.

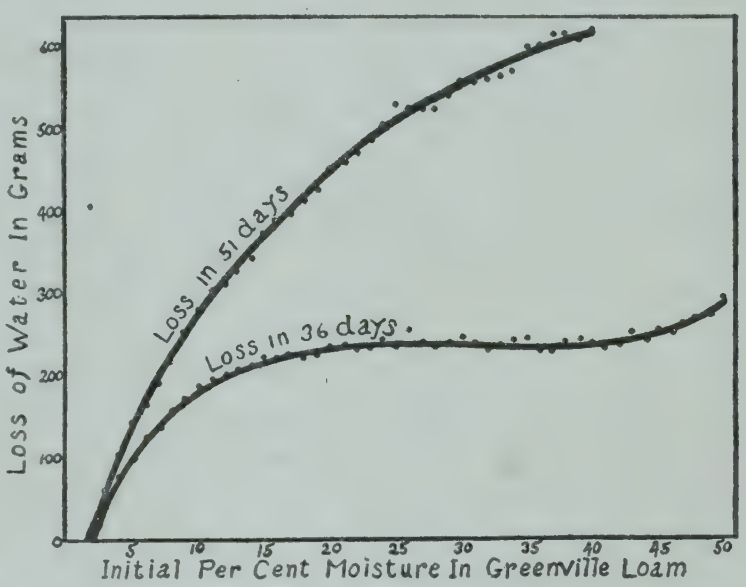

FIG. 1.—Evaporation from Greenville loam containing different initial percentages of moisture.

In some of the trials the free water lost more by evaporation than any of the wet soils, but usually there was a greater loss from soil which was completely saturated than from the free-water surface.

In each of these tests with Greenville loam, there seemed to be a number of more or less definite breaking points in the curves of loss. These indicate critical points where the moisture relations of the soil made rather sudden changes. A great deal of work will need to be done

under favorable conditions before these exact points of change can be determined.

In the summer of 1912 nine large galvanized-iron pans, or tanks, 5½ feet long by 1 foot wide by 3 inches deep were employed to give larger surfaces and deeper soil. The equivalent of 10 kgm. of dry Greenville loam was put into each tank and puddled with excess water to firm the soil and smooth its surface. Enough water in addition to the water already present—5.4 per cent—was added to make a series with 5 per cent intervals from 5.4 to 35.4 per cent. These tanks were set on the floor of the laboratory and were weighed on Monday, Wednesday, and Friday of each week. The losses were made up at the time of weighing. This study ran from June 12 to July 15, a period of 34 days.

The next year seven tanks were set up and run during the 81-day period from June 18 to September 6. This time 7.2 kgm. of Greenville loam, containing 2.6 per cent moisture, were used. Intervals of 5 per cent were again made, bringing the moisture content up to 7.6, 12.6, etc., as high as 37.6 per cent. Weighings were taken three times a week as in the first trial and water added to make up the evaporation loss. Table IV contains the results of the two trials.

TABLE IV.—*Effect of initial percentage of soil moisture on evaporation from Greenville loam in galvanized-iron tanks 5½ feet long and 1 foot wide*

1912		1913		Average of 2 tests.	
Moisture.	Loss in 34 days.	Moisture.	Loss in 34 days.	Average moisture.	Loss in 115 days.
Per cent.	*Gm.*	*Per cent.*	*Gm.*	*Per cent.*	*Gm.*
5.4	3,744	7.6	10,710	6.5	14,454
10.4	7,575	12.6	17,530	11.5	25,105
15.4	9,518	17.6	19,275	16.5	28,793
20.4	9,200	22.6	20,395	21.5	29,595
25.4	10,128	27.6	20,300	26.5	30,428
30.4	10,340	32.6	19,870	31.5	30,210
35.4	11,045	37.5	19,990	36.5	31,035

Table IV shows that the loss was rapid to 16.5 per cent and then slow. Of course, the wide intervals prevented locating exact points, but in general this test corroborates rather closely those already reported for loam.

SAND, CLAY, AND MUCK

During the period from December 26, 1913, to January 3, 1914, similar tests were made with sand, clay, and muck. One hundred gm., dry weight, of sand and clay and 50 gm. of dry muck were used in the same kind of tin plates as those used in the other trials. All percentages were run in triplicate, the sand for each percentage from 1 to 33, the clay for each percentage from 1 to 55, and the muck for each 20 per cent interval from

20 to 240 per cent. The muck consisted almost entirely of vegetable mold gathered from accumulated pockets in the brush swales of Logan River. It was known to have high water-holding capacity from previous experiments.

The pans were kept several inches back from the edge of the tables on which they rested. This almost entirely eliminated the effect of air currents which had, in previous tests, caused some variations in the rows of pans set close to the edge. The loss was made up each day after the weighing. The winter losses were much higher in this case than with the loam previously reported.

Figure 2 gives the results for sand and shows an increase in evaporation as the initial moisture is increased, up to 33 per cent. The most rapid increase is up to about 7 per cent. Above this point the increase in loss is not so great.

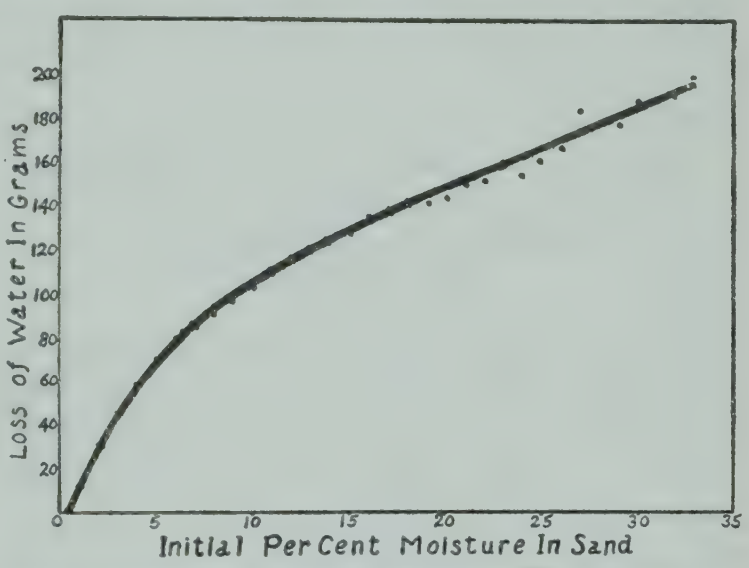

FIG. 2.—Evaporation from sand containing different initial percentages of moisture.

Figures 3 and 4 give the curves for clay and muck. The results for clay show a more gradual ascent in the curve, and a higher point before there is any break, than do the results for sand. This might have been expected from the great water-holding power of the clay. Muck with high percentages and differences at greater intervals produced about the same kind of curve as did loam.

More tests are necessary, however, to establish points as nearly exact as was done in the case of loam.

HUMIDITY OF THE AIR

To study the effect of a saturated atmosphere on evaporation, a set of wet soils in Petri dishes was placed in an air-tight copper germinator. Forty gm. of dry soil were put in each of these dishes, which were about 3 inches in diameter. Duplicate vessels were made up with soil for each 1 per cent from 1 to 35, and for each 2 per cent from 35 to 45 per cent of moisture. They were then set on the shelves of the germinator, which is a hollow box 22 inches wide, 37½ inches deep, and 47½ inches long. The whole was surrounded with a water jacket, except at the doors, which were made of two panes of glass inclosing a dead-air space about half an inch thick. The perforated copper shelves were covered with

cheesecloth and connected by a perpendicular wick to a water container on top. The wick kept all the cheesecloth covers saturated. To make sure no vapor escaped at the door, pieces of cloth kept constantly dripping were hung in front of the door. Tested thermometers showed throughout the experiment an almost constant temperature of 19.5° C., about one degree lower than that of the laboratory. Each day for 10 days the vessels were weighed to 0.01 gm. and made up by adding the water lost. The lower percentages, however, gained and were left at the wetness reached, which was about 7 per cent.

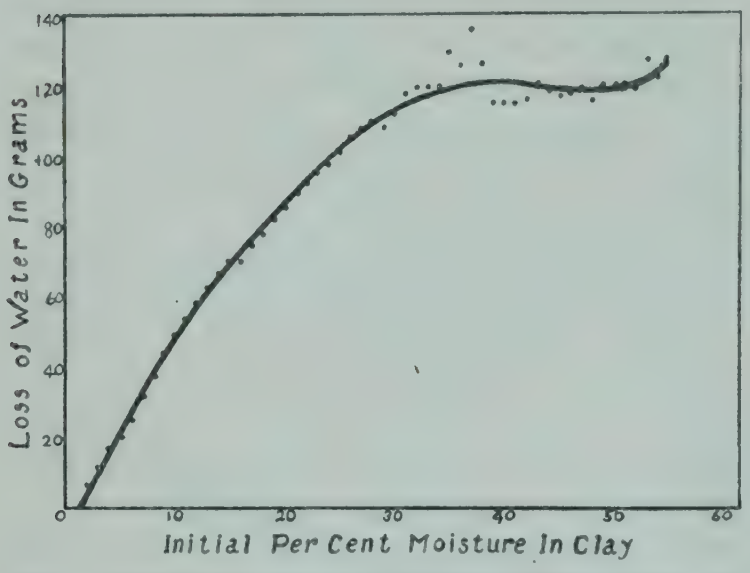

FIG. 3.—Evaporation from clay containing different initial percentages of moisture.

Fairly comparable with this test was another in which the same vessels, made up as before, were surrounded with cheesecloth 24 inches high to prevent drafts and to maintain a uniform temperature. Possible variations were eliminated by shifting the vessels in such a way that each occupied all sections of the cloth box some time during the 20 days of the experiment. The top was left open to permit vapor to escape upward. The relative humidity of the air was almost constantly at 76 per cent, whereas it was about 68 in the open laboratory. The temperature averaged 19° C. during the 20 days. Completely dried soil gained to 4 per cent, then remained constant. Weighings were made each day to the nearest 0.01 gm. and the losses made up as with the test in the germinator.

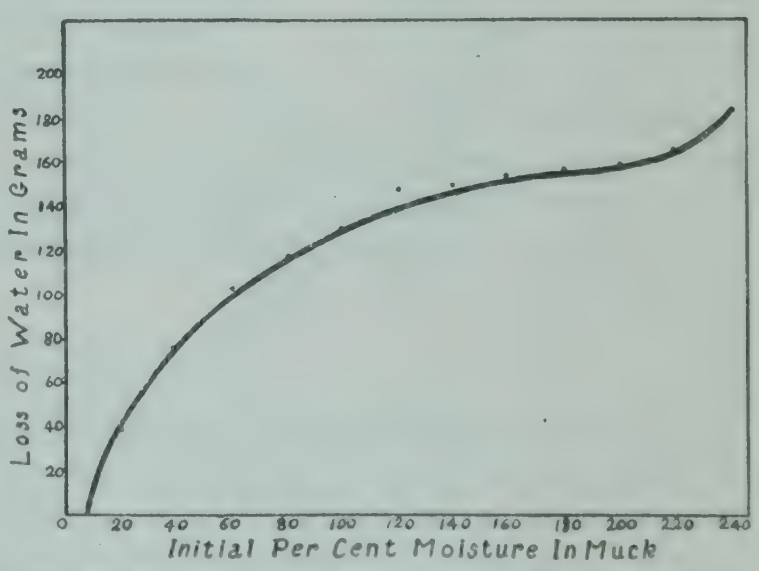

FIG. 4.—Evaporation from muck containing different initial percentages of moisture.

Figure 5 shows the losses for each 1 per cent in the saturated atmosphere of the germinator and for the slightly overmoist air of the cloth boxes.

Since the latter ran 20 days, the total losses have been divided by 2 in order to get the total loss for 10 days to make the figures comparable.

There is a decided difference in the results of the two experiments. The losses in the cloth boxes were nearly 20 times as great as those in the germinator, showing the enormous retarding effect of high humidity. Moreover, the open laboratory was somewhat drier and about one degree warmer than the air in the cloth boxes. No test was made in the open laboratory, but the losses would have been somewhat higher, since both a drier air and a higher temperature prevailed. This would, of course, further accentuate the already enormous differences.

WIND VELOCITY

As was suggested in the literature on wind velocity, King (9) has shown that vessels 20 feet from a windbreak lost 30 per cent less moisture by evaporation than vessels 30 feet distant. This was because the outer vessels were more exposed to air movement. Payne (13) shows nearly similar results, while Bowie (1) reports that with light winds and normal temperature there is an evaporation loss of 0.5 per cent for each mile of wind.

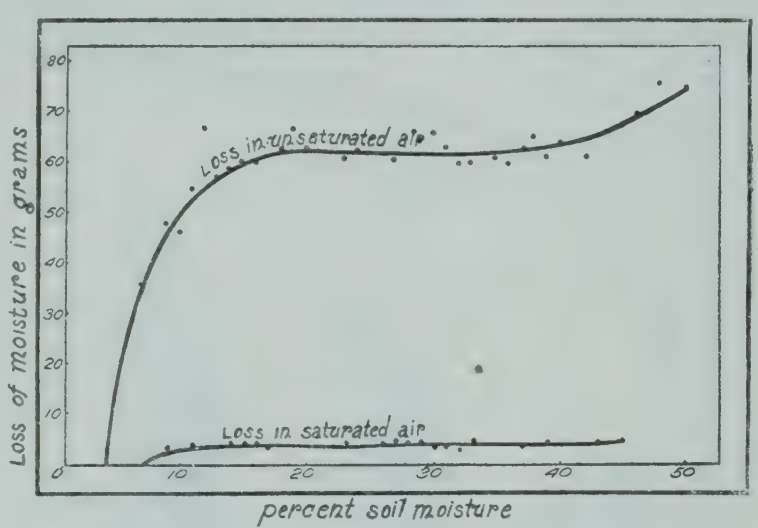

FIG. 5.—Loss of moisture from Petri dishes containing different percentages of soil moisture and kept in a saturated and unsaturated atmosphere.

In order to determine the effect of wind velocity on the rate of evaporation, a series of alleys 8½ inches wide and 70 inches long were arranged in such a way that the air could be made to pass through them at different velocities. The alleys were separated by oilcloth partitions 2 feet high; the air currents were made by electric fans placed in such positions that the desired velocities could be obtained. The velocity of air in each alley was measured with an anemometer placed at the end of the alley away from the fans.

The soils were contained in copper evaporimeters 6 inches in diameter. The loss each day was made up by adding water through a tube entering the evaporimeter at one side of the soil. In each alley there were five evaporimeters—one containing distilled water, one Greenville loam, and one each of quartz sand 0.25 mm., 0.5 mm., and 0.8 mm. in diameter.

Two tests were made in the experiment, one running continuously for 16 days with three wind velocities and another running continuously for

20 days with seven velocities. In each case there was an alley where the air was kept quiet. The combined results of the two tests are shown in figure 6. There is a rapid increase in evaporation with increased wind velocity at first; but after a velocity of about 10 miles per hour is reached, the increase in evaporation is slight. The water loss with the highest velocity was over four times that of the calm for the 16-day trial and nearly six times that of the calm in the 20-day test.

SUNSHINE

The general effect of sunshine in increasing the evaporation of soil moisture has long been known. The work of Seelhorst and Widtsoe on this subject has already been reviewed. In order to get more data an experiment was begun in the summer of 1913 at the Utah Agricultural Experiment Station. A spot on the college lawn just west of the main building was selected, because it was level and exposed to sunshine most of the day.

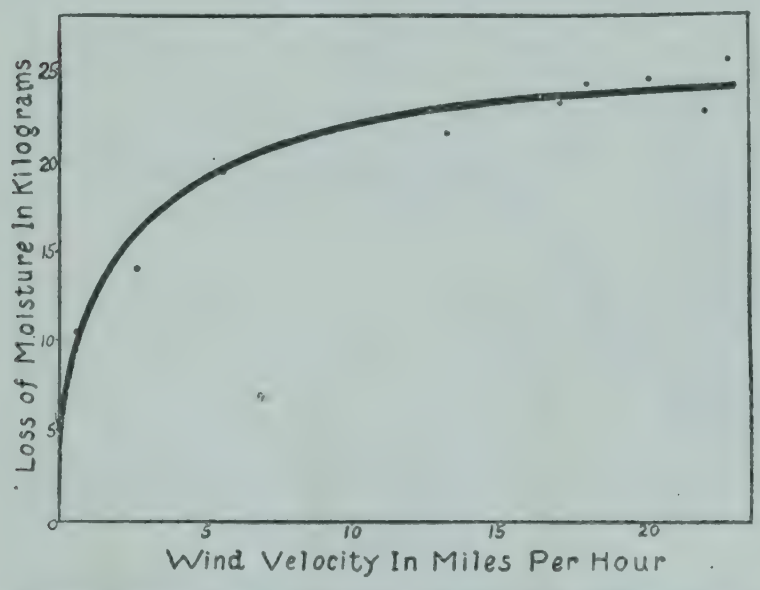

FIG. 6.—Evaporation of water from wet soils with different wind velocities.

Small tin plates containing 100 gm. dry weight of Greenville loam were prepared by puddling the soil and then drying, in order to get uniformity. They were then made up to the desired degree of wetness—5, 10, 15, 20, and 25 per cent, respectively. Each treatment was run in triplicate, making 15 pans to the set. One set was exposed to open sunshine, another was placed under a shade of cheesecloth 8 inches above the soil, and a third was shaded by a tight board cover also 8 inches above the soil. In each case the pans of soil were placed on a floor of boards and air was allowed to circulate freely over the soil beneath the cover.

The pans were each morning made up in the laboratory to the proper wetness and carried outside. Temperature readings were taken in the morning when the pans were carried out by laying a tested thermometer for a few minutes between the pans. A reading was taken at noon and another in the afternoon just before the pans were carried back into the laboratory. Another weighing of the pans showed the recorded loss due to evaporation. The pans were then left over night to be made up in the morning before placing outside.

Table V shows the average temperature for morning, noon, and afternoon. It is worth noting that the shade caused a reduction in temperature, complete shade causing a greater reduction than part shade.

TABLE V.—*Effect of shading on temperature and evaporation; temperature (° C.) average for 13 days*

Treatment.	Time.			Average.	Evaporation loss.
	Morning.	Noon.	Evening.		
					Gm.
Sunshine	30.2	41.3	34.2	35.2	554.8
Half shade (cheesecloth)	25.6	32.2	28.7	28.8	513.4
Shade (wood)	19.7	23.2	24.6	22.5	407.8

Table V shows the total loss in grams for each treatment. Figure 7 shows by graph the average temperature and the total loss with sunshine for part and for complete shade. It is noticeable that the evaporation losses decrease as shade increases.

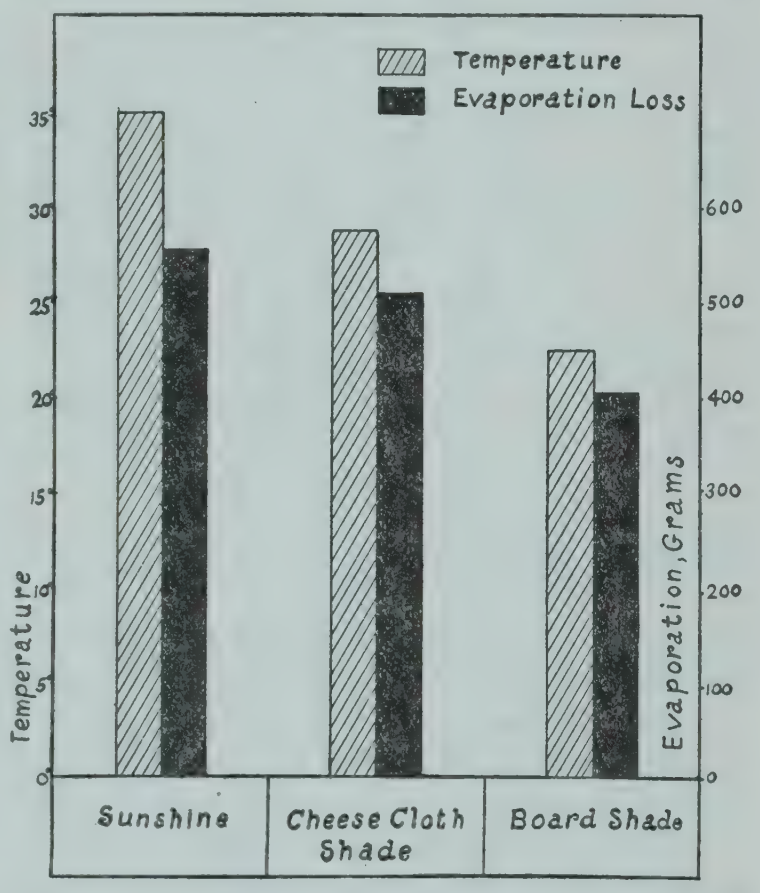

FIG. 7.—Loss of water from soil and temperatures in the sun and under cheesecloth and board shade.

TEMPERATURE

Temperature, relative humidity, and initial quantity of moisture are usually considered to be the most important factors in determining the intensity of evaporation. Perhaps temperature is most active. Fortier (5) shows that a reduction from 88° to 53° F. causes a corresponding reduction in evaporation loss of 85 per cent. These figures suggest the close relationship of evaporation to temperature. This important effect of temperature was made clear in a number of experiments where other factors were being studied.

To get some specific effects of temperature on the rate of evaporation of moisture from the soil, a number of large water baths were so arranged

that they could be kept uniform in temperature to within about one degree. Twenty-five gm. of soil were moistened and then put in flat-bottomed aluminum cans 7 cm. in diameter and 3 cm. deep, the cans being about one-fourth full. In order to bring the temperature of the soil quickly to the temperature of the water, these cans were allowed to float on water in the water baths. The baths were maintained at 20, 30, 40, 50, 60, 70, 80, and 90° C. It was almost impossible to maintain a uniform temperature when the bath was hotter than 90°; and when it was colder than 20° the evaporation was too slow to give noticeable results.

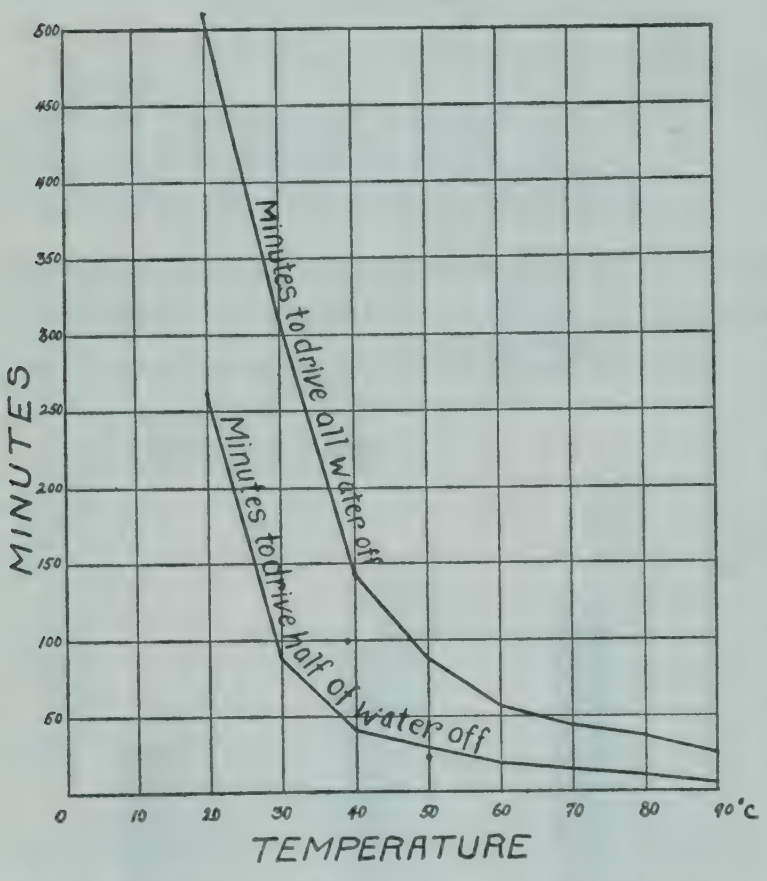

FIG. 8.—Time required at different temperatures to drive off half and all the water from Greenville loam containing 12 per cent moisture.

Two soils were investigated. The first was Greenville loam with an initial moisture content of 12 per cent of the dry weight of the soil and the second was a coarse sand with 20 per cent of initial moisture on the dry basis.

Hundreds of weighings were made to determine the rate of loss with each temperature. These weighings gave the results contained in figures 8 and 9.

The loam containing 12 per cent of moisture required 265 minutes at 20° C. to lose half of its moisture and 510 minutes to become practically dry. At 30°, 89 and 312 minutes were required to make it half and completely dry, respectively; at 40°, 46 and 143; at 50°, 23 and 88; at 60°, 17 and 56; at 70°, 12½ and 45; at 80°, 9½ and 38; and at 90°, 7 and 27.

In sand containing 20 per cent of moisture, the number of minutes required to become half and completely dry respectively at the various temperatures was as follows: At 20°, 315 and 819; at 30°, 90 and 240; at 40°, 45 and 100; at 50°, 30 and 72; at 60°, 13 and 35; at 70°, 9 and 24; at 80°, 6 and 18; and at 90°, 4½ and 13 minutes.

In the loam containing 12 per cent of water as an average, it required nearly three times as long to drive off the last 6 per cent of water as it did for the first 6. In the sand containing 20 per cent, it required more than

twice as long to evaporate the last 10 per cent as it did the first. In each case the last water that was driven off was the hygroscopic moisture. This probably accounts for the greater time required.

SIZE OF SOIL PARTICLES

Since evaporation takes place almost entirely at the surface, the rate of capillary movement directly affects this form of water loss. Because the size of soil particles and the porosity of the soil influence capillary movement, they indirectly affect evaporation. Principi (14) says that materials having the greatest pore space permit greatest evaporation. Wollny (21) reports no capillarity with particles more than 2 mm. in diameter, and also an increase as the particles get finer, though in clay the movement is slow. Losses from below the surface take place, according to Buckingham (2) by diffusion and vary with the square of the porosity of the soil.

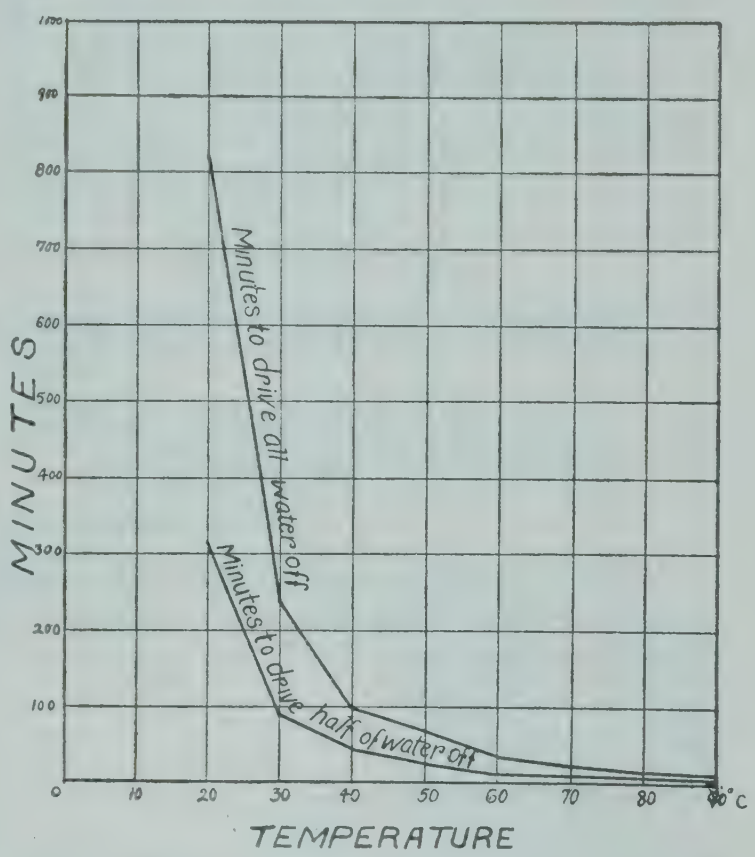

FIG. 9.—Time required at different temperatures to drive off half and all the water from sand containing 20 per cent moisture.

On account of the great difficulty in interpreting the results connected with this phase of the evaporation problem when ordinary soils are used, most of our work has been done with sands having grains of different diameters. The early experiments were conducted with river sands and gravels; but because of greater ease in obtaining uniform size of particles and because of lesser influence due to the composition of the materials, most of the later work was done with graded quartz sand.

In all trials except those in saturated atmosphere beneath the soil, these tests were made in the copper evaporimeters with free water maintained from about 1 to 3 cm. below the surface of the soil, the distance varying in different experiments. At weighings, which occurred about a week apart, the vessels were made up to their original weight.

The results of these tests are presented in figures 10, 11, and 12.

An examination of figure 10 shows a gradual increase in evaporation as the size of particles decrease. There are a number of irregularities probably caused by the difficulty in getting a uniform surface on all the evaporimeters. It will be noted that in some cases the evaporation was greater from the wet sand than from water.

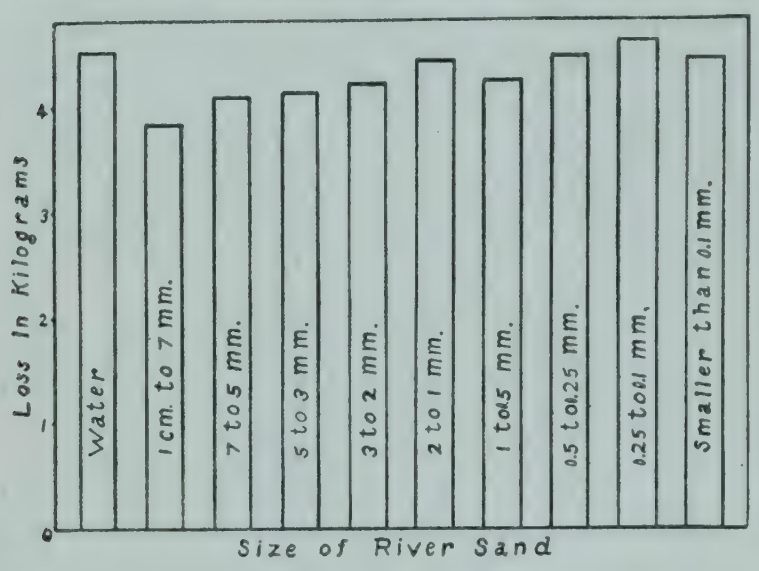

FIG. 10.—Evaporation of water in 66 days from sand of different sizes with a water table maintained 1 cm. below the surface.

In figure 11 results for three grades of pure quartz sand, for Greenville loam, and for water are shown. Here, as in figure 10, the finer grades lose more than the coarser.

Figure 12 includes five sizes of quartz sand, three sizes of river sand, and water. The differences are not marked, but are sufficient to bear out previous results in showing the greater evaporation from the surface of the smaller particles.

MULCHES

Wherever water storage in field soils is important, mulches are used to decrease the evaporation loss. Ridgaway (15) and Fortier (5) both indicate the great saving due to mulches made by stirring the topsoil or by adding covers of dry soil.

A number of laboratory experiments to study the effectiveness of different mulches when the effect of capillarity has been eliminated were conducted. In these experiments the different mulches were suspended above the water. The mulch was placed on wire gauze covered with cheesecloth or on perforated sheet metal to keep it about 1 cm. from the surface of the water in the lower part of the vessels. No water could evaporate except through the mulches.

FIG. 11.—Evaporation of water in 36 days from loam and sand of different sizes with a water table maintained 3 cm. below the surface.

A study of two sand mulches ½ inch and 1 inch deep, respectively, when placed where the sun would shine on them for half the day for 32 days shows a loss of 57 gm. for a mulch 1 inch deep, 60 gm. for a mulch ½ inch deep, and 155 gm. for the cheesecloth with no dry soil over it. Thus the shallower mulch lost but little more than 5 per cent more than the deeper. The check in which the water evaporated through the cloth and gauze lost nearly three times as much water as that from the mulches. This bears out Fortier's findings in regard to the effectiveness of mulches, but not in regard to the relative value of deep and shallow mulches.

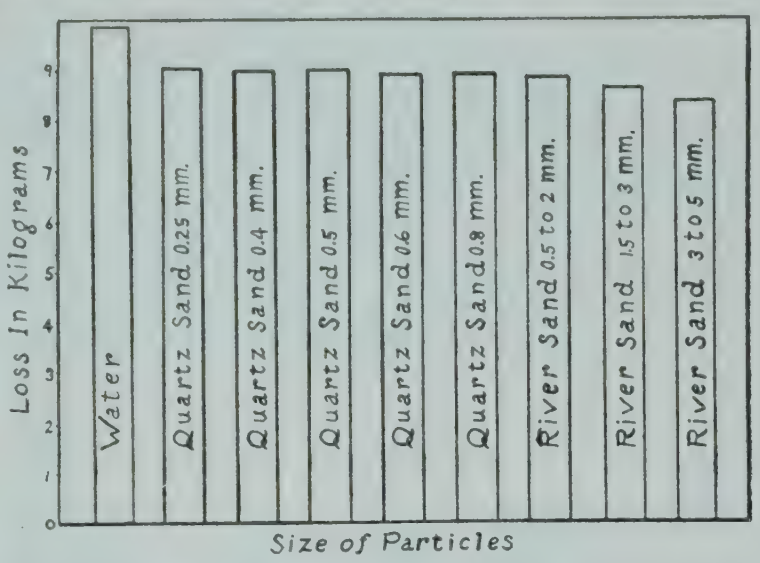

FIG. 12.—Evaporation of water in 115 days from quartz and river sand of different sizes with a water table maintained 3 cm. below the surface.

Figure 13 gives the results of the evaporation from 1-cm. mulches of river sands varying in size from 0.1 mm. to 7 mm. for 40 days. The loss is somewhat greater through the smaller sands. When, however, the sizes are larger than 1 mm., the variations in loss are irregular and inconclusive. The mulches prevented over half the evaporation that occurred from free water.

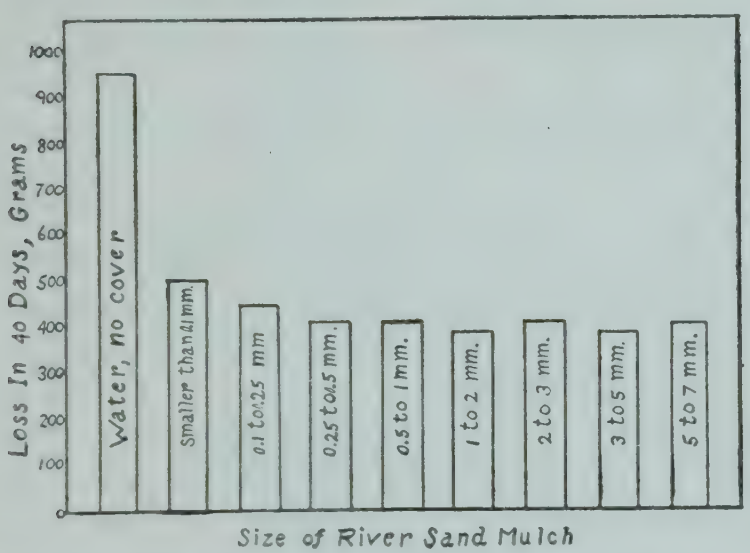

FIG. 13.—Loss of water from glasses having dry mulches of sand of various sizes suspended above free water.

With perforated aluminum lids 6 cm. in diameter and with a mulch nearly 2 cm. thick, a more exhaustive experiment was conducted. It was similar to the above, except that quartz sands were used for the smaller sizes and that tests with muck, clay, loam, and straw were also run. This experiment continued for a period equivalent to 180 days, the weighings being made at about 10-day intervals. Results of this experiment are shown in figure 14. Of the mulches the greatest loss was through the muck, followed by loam, clay, and straw in order, with the sand mulches

having the least evaporation. The sands show somewhat the same results as were found in the experiments mentioned above, except that there is a tendency for the losses to increase with increasing size of soil particles when the particles are more than 2 mm. in diameter.

COMPACTING THE SOIL

A set of six galvanized-iron cans 13 inches deep and 11 inches in diameter with an opening at the bottom through which water could be added from below to maintain the soil at a constant moisture content were filled with soil. Two sections of the soil at various depths were compacted in order to determine the effect of compacting on evaporation. The cans contained 12 inches of Greenville loam made up to about 15 per cent moisture. In can 1 the surface 2 inches were compacted; in can 2 the second 2 inches; and so on, until in can 6 the bottom 2 inches were compacted. The packed layers contained 20 per cent more soil in a 2-inch layer than a similar volume of the loose soil. These cans were weighed weekly for seven weeks and the loss made up through the side tubes at the bottom, which were kept closely stoppered except while water was being added.

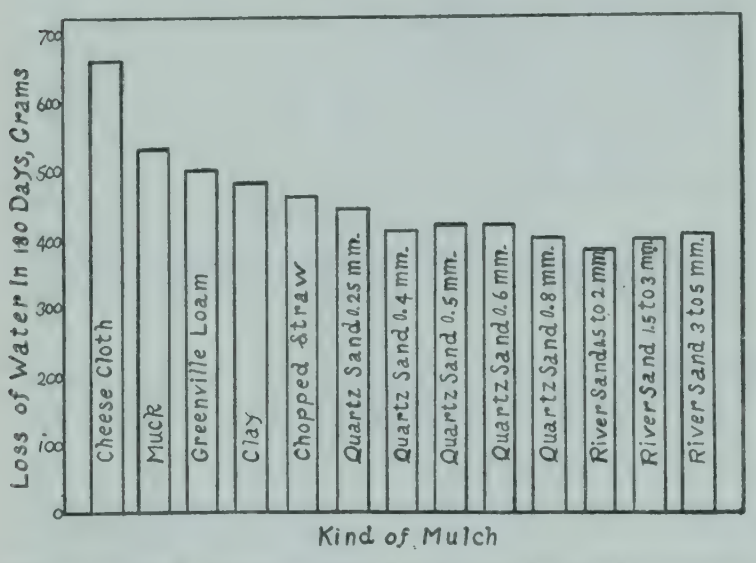

FIG. 14.—Loss of water in 180 days from glasses having dry mulches of various kinds suspended above free water.

Table VI shows that cans 1 and 2, compacted in the first and second 2-inch layers, respectively, lost much more heavily than the cans in which the packed layers were farther from the surface.

TABLE VI.—*Loss of moisture from cans of soil containing a 2-inch section compacted at various depths*

Can No.	2-inch section compacted.	Total loss.
		Gm.
1	Top	1,205
2	Second	1,045
3	Third	885
4	Fourth	870
5	Fifth	880
6	Bottom	805

Compacting the surface caused a marked increase in the loss; packing the second 2-inch section also increased the loss, but only about half as much. Compacting below 4 inches affected evaporation little if any.

METHOD OF APPLYING WATER

An important thing to know where irrigation is practiced is the effect on evaporation of applying water in different ways. Fortier (5) and Widtsoe (19) indicate that a great saving results from applying water in deep furrows or by subirrigation in which the water is added some distance below the surface.

In the summer of 1912 a study with soils 12 inches deep was conducted. Cylindrical vessels 11 inches in diameter and 13 inches deep were filled to within an inch of the top with Greenville loam. An equivalent of 10 kgm. of dry soil was used and made up with moisture ranging from 5.4 to 35.4 per cent in 5 per cent intervals. Weighings were made on three days weekly—usually Monday, Wednesday, and Friday. The losses were made up by adding water through spouts which entered the bottom of the cans and which were kept closed except while water was being added. Thus, the water had to move through 12 inches of soil, rather compact and unstirred, and evaporated from a small surface. Parallel to this test and used as a companion were the large galvanized-iron pans already described under the initial-quantity study. Here the soil was only about 1½ inches deep. To these shallow tanks the water was applied at the surface. The two trials were parallel throughout. The same percentages of moisture were used, weighings were made at the same time, they were run the same period, and the same kind of soil was used. A comparison of the two sets of results may be interesting, as they show the effect ot different methods of applying water.

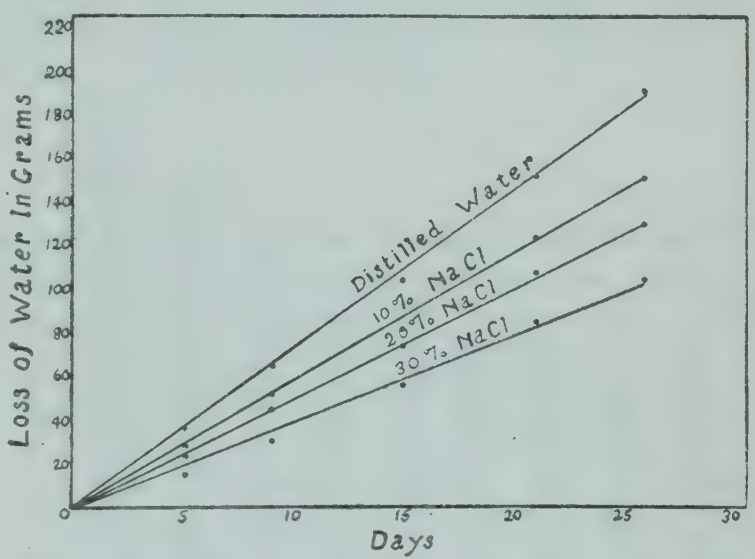

FIG. 15.—Evaporation from distilled water and from sodium-chlorid solutions of different concentrations.

Table VII gives the comparative data. In this table it may be noted that the losses at low percentages were somewhat more rapid from the shallow tanks, but that the deeper cans tended to lose more at the higher percentages. These had no free water exposed on top, while the wetter soils in shallow pans did. As already pointed out, the wet soils often

lost more than the free-water surfaces. The deeper soils were also more uniformly wet. Capillarity doubtless played a part in this experiment.

TABLE VII.—*Comparison of evaporation from large areas and shallow soil with small areas and deep soil, 10 kgm. of Greenville loam being used in each case*

Soil moisture.	Shallow tanks.			Deep cans.		
	Surface area.	Total evaporation.	Evaporation for each square foot of surface.	Surface area.	Total evaporation.	Evaporation for each square foot of surface.
Per cent.	*Sq. feet.*	*Gm.*	*Gm.*	*Sq. feet.*	*Gm.*	*Gm.*
5.4	5.42	2,935	542	0.66	270	409
10.4	5.42	6,100	1,125	.66	440	666
15.4	5.42	7,770	1,434	.66	700	1,066
20.4	5.42	7,405	1,366	.66	920	1,395
25.4	5.42	8,299	1,531	.66	1,010	1,530
30.4	5.42	8,415	1,553	.66	995	1,500
35.4	5.42	8,985	1,658	.66	1,270	1,939

SOLUBLE SALTS

The effect of dissolved salts in reducing the vapor tension, and consequently the evaporation of solutions, is well known. The action of these salts in the soil on evaporation, however, is not so clear, since secondary factors may be introduced. In the ordinary agricultural soil the concentration of soluble salts is not sufficient to have any marked effect on evaporation, but in the alkali soils of arid regions salts may be present in sufficiently high concentrations to affect the loss of moisture materially.

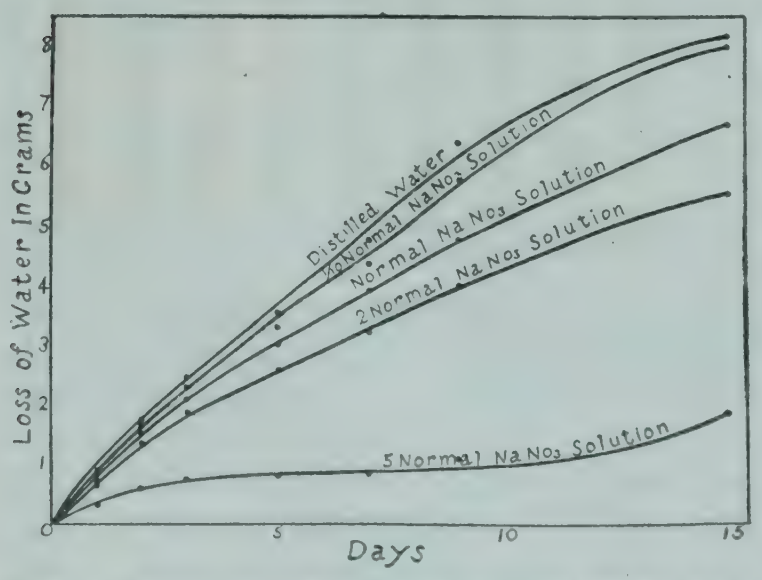

FIG. 16.—Evaporation from sand wet with distilled water and with sodium-nitrate solutions of differ ent concentrations.

With a view to determining some of the effects of salts on evaporation, a number of experiments were conducted. In the first, solutions of sodium chlorid of various concentrations without soil were investigated. The solutions were placed in glass tumblers, two tumblers being used for each treatment, and set in the open laboratory where evaporation could go on freely. The tumblers were weighted every few days and the loss made up with distilled water.

The results of this test are shown in figure 15, which brings out clearly the fact that as the concentration of the solution increases the evaporation decreases.

The second test was conducted in porcelain crucibles, each containing 10 gm. of quartz sand which had been wet with 4 c. c. of solutions of sodium nitrate ranging in concentration from a check solution containing no salt up to 5 times a normal solution. There were two crucibles for each concentration. The crucibles were placed under a bell jar in order to avoid air currents and to keep the humidity as uniform as possible over all the crucibles.

Weighings were made each day at first, and every two or three days later. The experiment was begun January 12 and continued till January 27, making a period of 15 days.

The results are given in figure 16, which shows a decrease of evaporation from the sand the same as when the solution of sodium nitrate is added. A third test was conducted in galvanized-iron cans, 11 inches in diameter and 13 inches deep, partly filled with Greenville loam to which sodium chlorid was added in different quantities, ranging from the control containing nothing to 7 per cent of the dry soil. The quantity of moisture that evaporated was added every three or four days through a tube near the bottom of the cans. In this way the surface of the soil was never disturbed, but there was a gradual accumulation of salts at the surface. The experiment ran from August 19 to September 25.

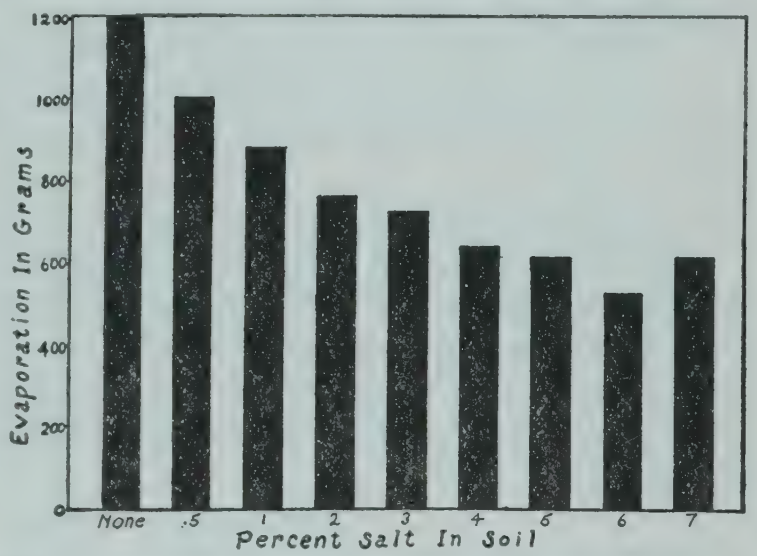

FIG. 17.—Evaporation of water from Greenville loam containing different quantities of sodium chlorid.

The results of the experiment are given in figure 17. There is a gradual decrease in the evaporation as the salt content of the soil is increased. The can with 7 per cent of salt lost slightly more than that with 6 per cent. This irregularity was doubtless due to the fact that considerable salt was crystallized at the surface of the soil in this can, consequently the real concentration of the solution was decreased; and it is the salt actually in solution that affects vapor tension.

From these experiments it seems clear that soluble salts in the soil decidedly decrease the evaporation of moisture if the concentrations are high, but the reduction is only slight for the solutions found in ordinary soils.

SUMMARY

(1) The conservation of soil moisture is one of the most important problems of agriculture, particularly in arid regions.

(2) One of the important factors involved in water conservation is evaporation.

(3) In this paper a study has been made of a number of the factors having to do with evaporation.

(4) Evaporation of moisture increases with the initial quantity in the soil. The increase is not so great with the higher percentages as with the lower, and there seems to be a number of critical points where the rate of loss changes rapidly.

(5) The rate of evaporation from a moist soil is very rapidly decreased as the humidity of the air is increased.

(6) Air currents greatly increase evaporation; but after about a certain wind velocity is reached, the rate of evaporation is only slightly increased by increasing the wind velocity.

(7) For the sizes investigated, evaporation was higher from the finer soil particles than from the coarser when both are completely saturated.

(8) Reducing the intensity of sunshine greatly reduces the rate of evaporation.

(9) Slight changes in temperature have a marked effect on evaporation.

(10) A thin mulch, if kept dry, is effective in reducing evaporation. Dry mulches, composed of fine particles, seem to be less effective than if composed of coarser particles.

(11) Compacting the surface of the soil increases evaporation.

(12) Dissolved salts in high concentrations reduce the evaporation of moisture from soils.

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MACROSIPHUM GRANARIUM, THE ENGLISH GRAIN APHIS

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Although the English grain aphis (*Macrosiphum granarium* Kirby) is widely disseminated throughout the United States and is a familiar pest of long standing, there are some interesting facts connected with its life history that heretofore have escaped observation. The object of this brief paper is primarily to put on record some details of life history and to discuss the interesting color variations in relation to the sexes.

SYNONYMY

Macrosiphum granarium seems to have been described first by William Kirby (1, p. 238, footnote)[2] in 1798. The description verbatim and complete is as follows:

> Possibly this may be the *Aphis avenae* of Fabricius: but as he has given no description of it, I cannot be positive; I shall therefore describe it under the name of *A. Granaria*, viridis, cauda biseta, setis geniculisque pedum nigris.
>
> Aphis avenae, *Fab. Sp. Ins.* ii. p. 386. n. 17. *Gmel.* tom. 1. part IV. p. 2206. n. 52. *Vill. Ent. Eur.* 1. p. 551. n. 50?
>
> Caput falvidu, uti antennarum articulus primus. Oculi nigri. Abdomen obovatum cauda aculeata. Pedes lividi, tarsis geniculisque nigris.
>
> Habitat in *tritici* et *hordei* spicis, aveneque paniculis.

Although meager, the foregoing description agrees with the species known to entomologists by this name. It can hardly be construed as a description of *Aphis avenae* Fab., as has been supposed by some authors. Curtis (3, p. 504) redescribed what he considered Kirby's species in the

[1] The writer wishes to acknowledge his indebtedness to Mr. T. H. Parks, lately of the Bureau of Entomology, for his assistance in conducting breeding experiments in 1909 at La Fayette, Ind., The observations upon which this paper is based were made at Richmond, Ind. (1907–8), La Fayette, Ind. (1909–1912), and Charlottesville, Va. (1915). Mr. J. J. Davis, of the Bureau of Entomology, kindly consented to prepare the synonymy.

[2] Reference is made by number to "Literature cited," p. 480.

Journal of the Royal Agricultural Society and again in his "Farm Insects" (6, p. 289). While there are minor points in Curtis's description and figures which seem to disagree, as a whole they apply quite well for *M. granarium* auct., and it is reasonably certain that he had this species before him when he made his description.

In 1843 Kaltenbach (2, p. 16) described *Aphis cerealis*. Pergande (11, p. 13–23) considered this to be distinct from *M. granarium*, basing his opinion largely on the presence or absence of abdominal maculations. However, this character is unreliable, as has been proved in breeding experiments where individuals showing all degrees of abdominal markings and some without the faintest trace of maculations were reared from the same mother. Most European authors now consider the two species *M. granarium* and *A. cerealis* as synonyms, and on inquiry the following replies have been received from the respective eminent European aphidologists. Under date of February 15, 1913, Prof. Fred W. Theobald writes: "I look upon *cerealis* and *granarium* as the same. I can see no difference." Under date of January 17, 1913, Mr. P. van der Goot writes as follows: "*M. granarium* and *M. cerealis* I must consider as one species." Dr. G. del Guercio has the following to say in a letter dated December 13, 1911:

> I have examined the specimens of *Macrosiphum* or *Siphonophora granariae*. They show some differences on which it may be possible for us to distinguish certain forms, which, however, as far as I am concerned, could never be considered varieties, let alone species. Fundamentally the *Siph. granariae* there (in America) is the *Siph. cerealis* here, and both in fact, secondary differences aside, are the same species. Your specimens have the antennæ a little longer than the body, while in our forms, at least those of Italy and of the European basin of the Mediterranean, the antennæ are shorter than the body. Buckton [7, p. 114–119, pl. 6], in his first volume, gives a good representation of *Siphonophora granariae*. (Free translation from the Italian.)

In 1849 Walker (4, p. 45–46) described this species as *Aphis avenae* Fab. The species was transferred to the genus Siphonophora by Koch (5, p. 186–187) in 1857, to Nectarophora by Oestlund (8, p. 82) in 1887, and finally to the genus Macrosiphum by Schouteden (10, p. 113–117) in 1901. In 1905 Kirkaldy (12, p. 132) proposed the name *M. avenivorum* for *M. granaria* Buckton, nec Kirby, and this must now be considered a synonym of *M. granarium*.

The synonymy as it now stands is as follows:

Macrosiphum granarium Kirby.

Aphis granaria Kirby, 1798, *in* Trans. Linn. Soc. [London], v. 4, p. 238.
? Aphis hordei Kyber, 1815, *in* Mag. Ent. [Germar], 1798, *in* v. 1, pt. 2, p. 211, *nomen nudum*.
Aphis cerealis, Kaltenb., 1843, Monog. Fam. Pflanzenläuse, p. 16.
Aphis granaria Curtis, 1845?, 1860, Jour. Roy. Agr. Soc., England, v. 6, p. 504; Farm Insects, p. 289.
Aphis avenae Walker (nec Fab.), 1849, *in* Ann. and Mag. Nat. Hist., s. 2, v. 3, p. 45–46.
Bromaphis Amyot, 1847, *in* Ann. Soc. Ent. France, s. 2, v. 5, p. 479.
Siphonophora cerealis Koch, 1857, Monog. Pflanzenläuse, p. 186–187.
Siphonophora granaria Buckton, 1876, Monog. Brit. Aphides, v. 1, p. 114–119, pl. 6.
Nectarophora granaria Oestl., 1887, *in* Geol. and Nat. Hist. Survey Minn. Bul. 4, p. 82.
Macrosiphum granarium Schout., 1901, *in* Ann. Soc. Ent. Belg., t. 45, p. 113–117.
Macrosiphum avenivorum Kirkaldy, 1905, *in* Entomologist, v. 38, p. 132.

DISTRIBUTION IN THE UNITED STATES

M. granarium undoubtedly occurs throughout the United States wherever the small grains are cultivated. The map (fig. 1) indicates localities from which the Bureau of Entomology has records of occurrence. It will be noted that there are 10 States from which the Bureau has no records, though *M. granarium* undoubtedly occurs in those States.

FOOD PLANTS OF THE APHID

This aphid does not confine itself exclusively to its well-known host plants, the small grains, but will live and thrive on a number of the wild and cultivated grasses.

Riley (9) listed *Agrostis vulgaris* [*alba*], *Bromus secalinus*, *Dactylis glomerata*, and *Poa pratensis* as host plants. Besides the plants just

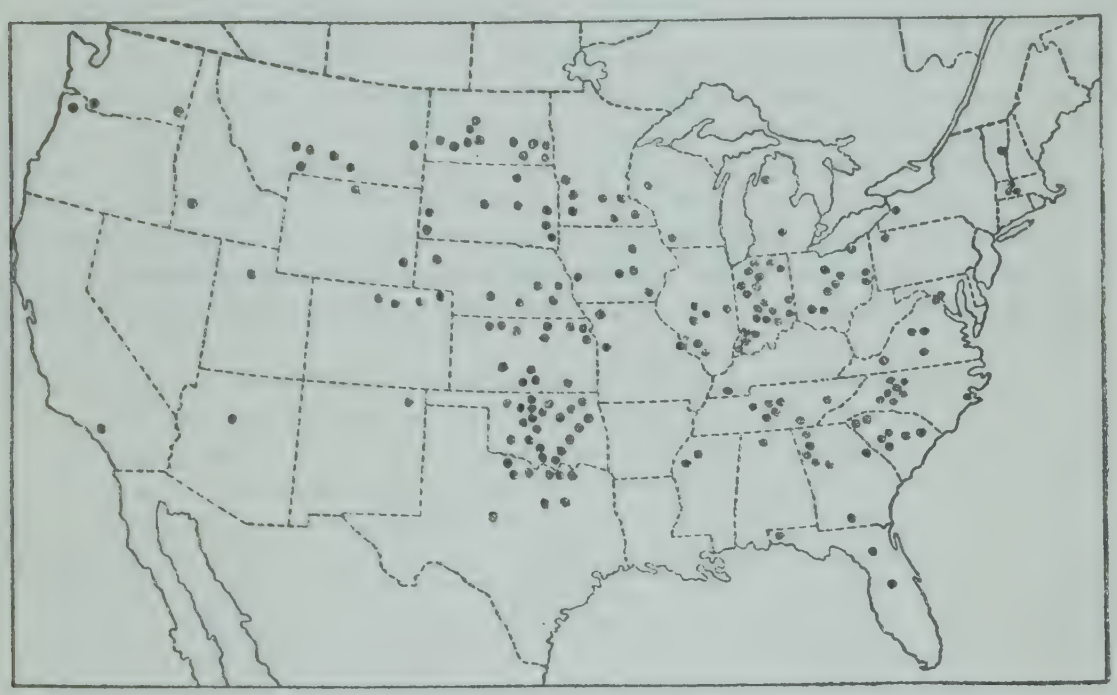

FIG. 1.—Map showing the distribution of *Macrosiphum granarium* in the United States as indicated by records on file in the Bureau of Entomology, 1916.

mentioned, the late F. M. Webster recorded it, in notes on file in the Bureau of Entomology, as breeding on heads of timothy (*Phleum pratense*) at Mitchell and La Fayette, Ind., in 1889, and in 1890 he recorded it as feeding on corn (*Zea mays*) at La Fayette, Ind. In 1904 Pergande (11) recorded *Elymus* sp. as a host.

A series of experiments conducted at La Fayette, Ind., in 1909, showed that *M. granarium* will breed and thrive in confinement upon the following grasses: *Bromus commutatus* (?) [*racemosus*], *B. secalinus*, *Elymus* sp., *Festuca duriuscula* [*ovina*], *F. heterophylla*, *F. pratensis* [*elatior*], *F. tectorum*, *Juncus tenuis*, *Lolium italicum*, *Poa compressa*, and *P. pratensis*. *M. granarium* was found to breed freely in confinement upon *Eleusine indica* and foxtail (probably *Chaetochloa glauca*) at Richmond,

Ind., in 1908. Most of the grasses in the experiments at La Fayette, Ind., were obtained from the experimental plots of the Purdue Experiment Station.

Other members of the Office of Cereal and Forage Insect Investigations have recorded the following hosts: *Bursa bursa-pastoris*, Nashville, Tenn. (G. G. Ainslie); *Syntherisma sanguinale*, North Vernon, Ind., 1908; *Echinochloa crus-galli*, Princeton, Ind., 1908; and *Hordeum pusillum*, Salisbury, N. C., 1909 (R. A. Vickery).

DESCRIPTIONS OF THE FORMS

STEM MOTHER

A number of stem mothers were secured at La Fayette, Ind., in the spring of 1911; but as the writer was absent at the time, the description was written by Mr. J. J. Davis. There was some slight variation in color of the adults, some having a somewhat yellowish tinge.

Body, including thorax, slightly darker than apple green on the dorsum; venter very slightly pruinose, giving it a silvery or whitish color. Eyes dark red. Head pale brownish. Antennæ (Pl. 34, F): I and II concolorous with head, but with slight duskiness; III, IV, V, and VI black. Beak pale brownish at base and the last two segments black. Tip of beak just reaching second coxæ. Legs: Basal half of femora pale greenish, distal half dusky to black; tibiæ pale, with slight brownish tint, the distal end black; tarsi black. Cornicles black; style pale whitish, with slight greenish tint.

Measurements made from four individuals immediately after mounting in balsam:

Length of body.	With style.	Width.
Mm.	*Mm.*	*Mm.*
2. 27	2. 59	1. 21
2. 63	3. 02	1. 47
2. 39	2. 39	1. 12
2. 22	2. 50	1. 24

Cornicles, 0.39, 0.515, 0.408, and 0.51 mm., respectively. The antennal measurements are given in Table I.

TABLE I.—*Length (in millimeters) of antennæ of the stem mothers of Macrosiphum granarium*

No.	Specimen No.						
	1	2	3	4	5	6	7
I	0. 106	0. 106	0. 12	0. 11	0. 10	0. 155	0. 155
II	. 07	. 07	. 08	. 07	. 066	. 06	. 06
III	. 43	. 44	. 53	. 53	. 435	. 586	. 586
IV	. 195	. 185	. 30	. 28	. 15	. 31	. 30
V	. 24	. 23	. 28	. 30	. 23	. 337	. 337
VI base	. 11	. 12	. 12	. 13	. 12	. 14	. 14
VI filament	. 31	. 34	. 34	. 39	. 32	. 42	. 44
Total	1. 571	1. 491	1. 77	1. 81	1. 421	2. 008	2. 018

SUMMER FORMS

The following description is taken from Pergande (11):

Apterous female. [Pl. B, 2.] Length 2.4 to 2.8 mm.; fusiform, broadest near the base of the abdomen. Frontal tubercles large, diverging at the apex, as usual, in this genus; antennæ bristle-shaped, as long or slightly longer than the abdomen; joint six, including the spur, longer than joint three; generally there are one or two small, circular and projecting sensoria near the base of the third joint; all of the joints are very sparsely beset with short and stiff bristles which are rarely slightly clavate. The nectaries are long and reach beyond the tip of the abdomen, though rarely beyond the tip of the tail; they are cylindrical, tapering, becoming again slightly stouter toward the end. The tail is rather long and stout, curved upward, and about two-thirds the length of the nectaries, lanceolate, and more or less distinctly constricted about the middle; it is densely covered with acute, minute points and furnished each side of its terminal half with three, backward-curved, long bristles. The legs are long and provided with short, stiff, and simple hairs.

The color of the apterous female is yellowish-green, often slightly pruinose; frequently darker toward the end of the body; the head varying from yellow to brownish-yellow. The eyes are red to brown, while the tail varies from white to a distinct yellow. The antennæ, as a rule, are black, though sometimes the first joint may be yellow or the first three joints dusky. The terminal half or more of the femora, apex of the tibiæ, the tarsi, and the nectaries brown to black; the rest of the leg is yellow. The body is frequently marked with a brownish puncture or spot each side of the prothorax; sometimes there is a narrow dusky or black line, composed of minute spots, each side of the mesothorax and a dorso-lateral row of about five linear or rounded, blackish or dusky spots each side of the abdomen, which sometimes are extremely faint or even wanting. Occasionally there are also two additional small black or dusky spots between the nectaries. Lateral spots in front of nectaries black.

Winged migrant. [Pl. 33, A.] Expanse of wings 9 to 9.4 mm.; length of body 1.4 to 2.6 mm. Antennæ long, generally about one-third longer than the body; the third joint about one-third shorter than the sixth and provided along its exterior or posterior edge with from six to eleven more or less elevated, round sensoria along its basal third. The hairs of the various joints are similar to those of the apterous female, though sometimes one or the other may be distinctly clavate. The nectaries, tail, and legs in general appearance and size are very similar to those of the apterous form. The wings are almost twice the length of the body, while the venation corresponds very much to that of Aphis.

Color yellowish green to green; the mesothorax yellow and its lobes brown to black. Sometimes a small, oblique, dusky, subdorsal spot and a transverse pale dusky band may be observed on the prothorax. Head brown or brownish-yellow; eyes red to brown. Antennæ black, the first joint sometimes brownish-yellow externally. Nectaries black, the tail yellowish or greenish-yellow; sternal plate and lateral spot in front of wings black. The abdomen is marked with four or five small, transverse, blackish dorso-lateral spots and four black lateral spots in front of nectaries; the coloration of the legs is similar to that of the apterous female. Wings clear, the costa dusky, and the subcosta yellow; stigma yellowish, its inner margin dusky; veins yellowish-brown, changing to black toward the end.

It is probably well to state in this connection that the maculation of the abdomen is quite a variable character and that the cornicles are reticulate at the tip.

The following measurements (Table II) were made from specimens that had been mounted in balsam for over a year.

TABLE II.—*Length (in millimeters) of the antennæ of summer forms of Macrosiphum granarium*

WINGED VIVIPAROUS FEMALES (PL. 34, B)

No.	Specimen No.						
	1	2	3	4	5	6	7
I	0.09653	0.09653	0.09653	0.09653	0.09653	0.09653	.....
II	.06895	.06895	.06895	.07584	.06895	.08274	.....
III	.66192	.60676	.66192	.64813	.59986	.63434	.....
IV	.55849	.55849	.57128	.56539	.47575	.46886	.....
V	.38612	.38612	.44128	.44128	.38612	.35854	.....
VI	.12411	.13100	.12411	.13790	.12411	.11721	.....
	.77224	.....	.66192	.64813	.75845	.64813	.....
Total	2.66836	.....	2.62594	2.61320	2.50977	2.40635	.....
Cornicles	.42749	.....	.43438	.....	.37233	.....	.....
	.41370	.....	.44128	.....	.38612	.....	.....
Cauda	.30338	.....	.30338	.....	.27580	.....	.....

WINGLESS VIVIPAROUS FEMALES (PL. 34, D)

I	0.10342	0.11032	0.11032	0.11032	0.11032	.....	.....
II	.06895	.07584	.07584	.08274	.08274	.....	.....
III	.57918	.75228	.56539	.71708	.68950	0.62055	0.62055
IV	.35854	.35854	.42438	.52402	.52402	.42749	.44128
V	.31027	.28959	.33785	.37233	.38612	.31717	.33096
VI	.11712	.12411	.13790	.12411	.12411	.12411	.12411
	.56539	.53091	.68260	.70329	.68950	.60676	.62055
Total	2.00296	2.06159	2.33428	2.63389	2.60631	.....	.....
Cornicles	.....	.42749	.46196	.....	.59297	.....	.46886
	.....	.42749	.44817	.....	.59297	.....	.48265
Cauda	.....	.33096	.33096	.....	.41370	.....	.34475

SEXES

The following description of the sexes is from Sanderson (13):

Apterous oviparous female. [Pl. B, 5.] One specimen, 1.9 mm. long by 1 mm. wide; antennæ [Pl. 34, E] 2 mm., segments, 3, 0.50 mm.; 4, 0.35 mm.; 5, 0.30 mm.; 6, 0.10 mm.; 7, 0.50 mm.; cornicle, 0.43 mm.; cauda, 0.21 mm. Somewhat smaller than viviparous form. At first yellow, then turning green and darker green. Head light brown. Distal two-thirds of femora, tip of tibia, tarsi and cornicles black, antennæ black. Conspicuous horizontal black marking in pit of connexivum on either side, these being more or less connected by black lines on the sutures of the first six abdominal segments and coalescing to form a faint but distinct black spot on abdominal segments 4–6. Meta-tibia with numerous pores. [Pl. 34, C.]

Winged male. [Pl. 33, B.] Antennæ [Pl. 34, A] 2.8 mm.; segments, 3, 0.68 mm.; 4, 0.50 mm.; 5, 0.46 mm.; 6, 0.14 mm.; 7, 0.78 mm.; cornicle, 0.14 mm.; cauda, 0.14 mm.; wing 3.35 mm. long. The third antennal segment with 35 to 50 sensoria, the fourth segment with a row of 10 to 12 on basal two-thirds, about 10 large sensoria

on distal two-thirds of fifth segment, and usual large sensoria at tip of sixth and seventh segments. Similar to winged viviparous female, but reddish to reddish brown, with black markings on either side of dorso-meson of abdominal segments, especially on the seventh segment where the marking converges on the meson.

INTERMEDIATE FORM

One individual was found that contained only eggs. Her hind tibiæ were not swollen; nor did they have sensoria. The general color was the same as that of the oviparous female. It is not known whether she produced young previous to being mounted.

EGG

The egg is elliptical, 0.3 mm. in diameter and 0.7 mm. long. It is a pale yellow when first deposited, changing in a few days through different shades of green to black.

LIFE HISTORY AND HABITS

Eggs begin to hatch during the last week in March in the latitude of La Fayette, Ind., and continue hatching through the first week in April.

Eggs were obtained in Richmond, Ind., in the fall of 1908, but none hatched the following spring. Eggs were again secured in the fall of 1909 at LaFayette, Ind., though only one hatched from this lot. The mortality of the eggs is very high, but no definite cause can be assigned for this at present. The eggs of this species were placed in hibernation under apparently the same conditions as those of several other species of Aphididae, the latter hatching readily and *M. granarium* hatching very sparingly or not at all. From hundreds of eggs secured in the fall of 1910 only about 15 or 20 hatched. The eggs would remain plump until about time to hatch and would then shrivel. Eggs began hatching on March 24 in 1911.

As is common with Aphididae in general in this latitude, this species at La Fayette, Ind., reproduces parthenogenetically until October, when the sexes appear and eggs are deposited. The writer took adult males in the field on bluegrass at La Fayette in November, 1909, and young males were observed on rye and volunteer oats on the Purdue University farm in November, 1911. No oviparous females have been observed in the fields as yet, but the presence of the males indicates that the sexes occur normally on the small grains and on blue grass in the fall.

Mr. R. A. Vickery, of the Bureau of Entomology, stated that he has taken the sexes on wheat in Minnesota, but he made no mention of having obtained eggs or stem mothers. Sanderson (13) reared adults of both sexes indoors in Texas in 1903, although he made no record relative to the egg. It is doubtful whether eggs occur normally that far south.

Viviparous females have been carried through the winter out of doors in breeding cages at La Fayette, Ind., and at Charlottesville, Va., and have been found on the small grains throughout the fall, in the winter, and again in the early spring, so they doubtless pass the winter both in the egg and as viviparous females in the Northern States. It is doubtful whether eggs and stem mothers normally occur much south of latitude 35° unless it is in higher altitudes.

The aphids remain on the leaves of wheat and other small grains until the heads are formed and then cluster around the tender kernels, sucking the rich sap. Just before harvest, when the plant tissues become hard and tough, all immature individuals become winged and migrate to some of the grasses, where they remain until volunteer grain and fall wheat put in their appearance.

REARING CAGES

The same type of shelter and rearing cages were used as those previously described and figured by the writer (14).

GENERATION SERIES

The generation series were not started with stem mothers in any instance, as no eggs hatched until the spring of 1911. Since they had been carried through consecutive generation series for each of the three preceding years, it was thought unnecessary to continue longer. The series were started each year with the progeny of individuals that had survived the winter. In fact, they were started at the time the eggs of *Toxoptera graminum* began to hatch. It was found later that this was approximately the date of hatching for *M. granarium*.

The usual method that has been followed in the past by the Office of Cereal and Forage Insect Investigations in the generation rearing was adopted for this species—that is, the first born from each first born and the last born from each last born were isolated and daily records made as far as possible. Two partial generation series were carried through in 1907 and four complete ones in 1908 at Richmond, Ind.—that is, they were run either until the sexes appeared in the fall or until the work was interrupted by cold weather. Another generation series was carried through at La Fayette, Ind., in 1909 and one in 1915 at Charlottesville, Va. The writer thus has observations covering nearly 120 individuals. This is a comparatively small number, but since the observations cover practically four years the data should prove reliable.

Tables III and IV give in detail consecutive generations from one individual hatched March 27, 1908, at Richmond, Ind.

TABLE III.—*Line of generations of Macrosiphum granarium at Richmond, Ind., in 1908*

Date.	Temperature.		First-born generation series.																Last-born generation series.					
	Maximum.	Minimum.	First generation.	Second generation.	Third generation.	Fourth generation.	Fifth generation.	Sixth generation.	Seventh generation.	Eighth generation.	Ninth generation.	Tenth generation.	Eleventh generation.	Twelfth generation.	Thirteenth generation.	Fourteenth generation.	Fifteenth generation.	Sixteenth generation.	Seventeenth generation.	Second generation.	Third generation.	Fourth generation.	Fifth generation.	Sixth generation.	Seventh generation.
	°F.	°F.																						
Mar. 27	78	53	[a]B	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Apr. 18	63	55	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
19	70	50	2	B	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
20	68	39	2	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
21	68	33	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
22	79	35	2	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
23	79	52	2	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
24	71	60	2	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
25	74	61	4	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
26	80	53	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
27	67	42	2	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
28	54	38	1	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
29	46	36	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
30	47	33	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
May 1	56	29	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
2	47	35	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
3	55	37	1	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
4	50	41	2	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
5	52	49																						
6	76	49	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
7	63	41	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
8	49	41	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
9	65	42	4	2	B	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
10	73	44		2	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
11	76	45	2	4	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
12	80	60			0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
13	80	55	3	2	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
14	84	57																						
15	85	59	0	1	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
16	86	52	1	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
17	87	54	2	1	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
18	77	61	2	[b]D	2	B	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
19	68	54		....	4	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
20	76	54	....	....		0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
21	79	50	0	....	2	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
22	81	57	2	....	4	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
23	83	58	0	....	1	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
24	85	55	0	....	2	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
25	92	55	0	....		0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
26	86	66	2	....	1	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
27	88	57		....		2	B	....	....	....	....	....	....	....	....	....	....	....	....	B	....	....	....	....	....
28	90	57	0	....	0	4	0	....	....	....	....	....	....	....	....	....	....	....	....	0	....	....	....	....	....
29	90	62	0	....	0	4	0	....	....	....	....	....	....	....	....	....	....	....	....	0	....	....	....	....	....
30	78	62	0	....	0	1	0	....	....	....	....	....	....	....	....	....	....	....	....	0	....	....	....	....	....
31	71	55	0	....	D	3	0	....	....	....	....	....	....	....	....	....	....	....	....	0	....	....	....	....	....
June 1	64	43	0	....	....	1	0	....	....	....	....	....	....	....	....	....	....	....	....	0	....	....	....	....	....
2	76	43	0	....	....		0	....	....	....	....	....	....	....	....	....	....	....	....	0	....	....	....	....	....
3	70	52	0	....	....	2	0	....	....	....	....	....	....	....	....	....	....	....	....	0	....	....	....	....	....
4	82	61	0	....	....		0	....	....	....	....	....	....	....	....	....	....	....	....	0	....	....	....	....	....
5	84	50	0	....	....	5	0	....	....	....	....	....	....	....	....	....	....	....	....	0	....	....	....	....	....
6	87	55	0	....	....		0	....	....	....	....	....	....	....	....	....	....	....	....	0	....	....	....	....	....
7	90	60	0	....	....	1	2	B	....	....	....	....	....	....	....	....	....	....	....	0	....	....	....	....	....
8	86	64	0	....	....	4	4	0	....	....	....	....	....	....	....	....	....	....	....	0	....	....	....	....	....
9	78	64	D	....	....		0	0	....	....	....	....	....	....	....	....	....	....	....	0	....	....	....	....	....
10	77	54	....	....	....	1	2	0	....	....	....	....	....	....	....	....	....	....	....	0	....	....	....	....	....
11	72	43	....	....	....	0	1	0	....	....	....	....	....	....	....	....	....	....	....	2	....	....	....	....	....
12	82	51	....	....	....	2	0	0	....	....	....	....	....	....	....	....	....	....	....	0	....	....	....	....	....
13	88	59	....	....	....	2	2	0	....	....	....	....	....	....	....	....	....	....	....	0	....	....	....	....	....
14	70	58	....	....	....	3	1	0	....	....	....	....	....	....	....	....	....	....	....	0	....	....	....	....	....
15	64	45	....	....	....			2	B	....	....	....	....	....	....	....	....	....	....	0	....	....	....	....	....
16	74	30	....	....	....	2	0	2	0	....	....	....	....	....	....	....	....	....	....	3	....	....	....	....	....
17	82	47	....	....	....	1	2	1	0	....	....	....	....	....	....	....	....	....	....	1	....	....	....	....	....
18	90	65	....	....	....	0	0	3	0	....	....	....	....	....	....	....	....	....	....	0	....	....	....	....	....
19	91	66	....	....	....	4	1	8	0	....	....	....	....	....	....	....	....	....	....	0	....	....	....	....	....
20	89	65	....	....	....			0		....	....	....	....	....	....	....	....	....	....	0	....	....	....	....	....
21	89	65	....	....	....	1	0	4	0	....	....	....	....	....	....	....	....	....	....	2	....	....	....	....	....
22	91	64	....	....	....	2	D	2	0	....	....	....	....	....	....	....	....	....	....	1	....	....	....	....	....

TABLE III.—*Line of generations of Macrosiphum granarium at Richmond, Ind., in 1908*—Continued.

Date.		Temperature.		First-born generation series.																Last-born generation series.					
		Maximum.	Minimum.	First generation.	Second generation.	Third generation.	Fourth generation.	Fifth generation.	Sixth generation.	Seventh generation.	Eighth generation.	Ninth generation.	Tenth generation.	Eleventh generation.	Twelfth generation.	Thirteenth generation.	Fourteenth generation.	Fifteenth generation.	Sixteenth generation.	Seventeenth generation.	Second generation.	Third generation.	Fourth generation.	Fifth generation.	Sixth generation.	Seventh generation.
		°F.	°F.																						
June	23	93	68				2		4	4	B										0					
	24	94	67				2		5	6	0										0					
	25	79	52								0										0					
	26	79	49				0		2	4	0										0					
	27	88	48				D				0										0					
	28	89	52						1	4	0										0					
	29	81	60						2	4	0										0					
	30	82	54								0										1	B				
July	1	89	52						0	2	0										0	0				
	2	89	57						1	2	0										D	0				
	3	82	64						1	2	2	B										0				
	4	82	66						0	1	3	0										0				
	5	88	60						2	D	4	0										0				
	6	91	66						0		3	0										0				
	7	80	64						0		2	0										0				
	8	78	48						D		5	0										0				
	9	84	47								2	0										0				
	10	88	51								2	0										0				
	11	91	54								2	2	B									0				
	12	95	67								6	1	0									0				
	13	92	63										0									4				
	14	86	65								6	3	0									3				
	15	79	57										0									1	B			
	16	87	54								2	5	0									0	0			
	17	90	66								1	1	0									D	0			
	18	84	71										0										0			
	19	80	60										2	B									0			
	20	77	59								D	D	2	0									0			
	21	75	06										1	0									0			
	22	88	57											0									0			
	23	93	61										1	0									0			
	24	86	66										2	0									0			
	25	85	64										2	0									0			
	26	87	65										1	0									0			
	27	89	66										D	0									0			
	28	87	60											0									0			
	29	88	62											4	B								0			
	30	91	63											0	0								0			
	31	91	60											0	0								0			
Aug.	1	86	54											0	0								0			
	2	93	53											D	0								2			
	3	93	55												0								0			
	4	93	64												0								2			
	5	82	67												1	B								B		
	6	86	58												4	0							D	0		
	7	79	69												0	0								0		
	8	75	55												2	0								0		
	9	81	50												0	0								0		
	10	82	54												1	0								0		
	11	88	55												0	0								0		
	12	90	69												2	0								0		
	13	80	67												0	0								0		
	14	90	60												1	0								0		
	15	89	61												0	0								0		
	16	95	65												1	0								0		
	17	93	67												D	0								0		
	18	85	58													4	B							0		
	19	95	52													2	0							0		
	20	77	56													2	0							0		
	21	86	47													0	0							0		
	22	86	60													2	0							0		
	23	83	53													3	0							2		
	24	80	50													2	0							1		
	25	83	53													2	0							1		
	26	81	54													1	0							0		
	27	82	51													3	0							2		
	28	84	45													1	0							1		

TABLE III.—*Line of generations of Macrosiphum granarium at Richmond, Ind., in 1980*—Continued.

Date.	Temperature.		First-born generation series.																	Last-born generation series.					
	Maximum.	Minimum.	First generation.	Second generation.	Third generation.	Fourth generation.	Fifth generation.	Sixth generation.	Seventh generation.	Eighth generation.	Ninth generation.	Tenth generation.	Eleventh generation.	Twelfth generation.	Thirteenth generation.	Fourteenth generation.	Fifteenth generation.	Sixteenth generation.	Seventeenth generation.	Second generation.	Third generation.	Fourth generation.	Fifth generation.	Sixth generation.	Seventh generation.
	°F.	°F.																							
Aug. 29	93	51													1	0							1		
30	93	62													2	1	B						2		
31	95	54													2	1	0						1		
Sept. 1	90	50													0	0	0						0		
2	77	46															0								
3	78	37													2	2	0						0		
4	81	48													2	2	0						1		
5	86	50													0	0	0						1	B	
6	89	53															0								
7	74	46													1	0	0						0	0	
8	82	40													2	3	0						0	0	
9	90	44													D	0	0						D	0	
10	93	50														2	0							0	
11	94	51														1	5	B						0	
12	94	54														0	2	0						0	
13	89	57														1	4	0						1	
14	84	49														0	2	0						3	
15	84	56														3	2	0						2	
16	85	52														0	2	0						3	
17	87	50														0	1	0						0	
18	91	48														2	1	0						2	
19	93	50																0							
20	95	50														1	0	0						3	
21	95	51																0							
22	91	51														0	2	0						3	
23	90	53														0	1	0						1	
24	93	53														1	0	0						0	
25	92	51														0	2	0						1	
26	90	57														1	0	0						2	
27	87	54															0	0							
28	76	44														2	0	2						2	
29	53	34															0	0	B						
30	74	33														0	0	0						1	
Oct. 1	62	34														1	0	0						0	
2	57	25														1	0	0						1	
3	69	27														0	0	2						1	
4	79	27														0	0	1						0	
5	86	34														0	0	0						0	
6	83	39														0	0	0						0	
7	76	42														0	0	0						0	
8	60	41														0	0	2						0	
9	60	37														0	0	0						0	
10	65	45														0	0	0						0	
11	51	39														0	0	1						0	
12	59	25														0	0	0						0	
13	73	26														1	0	0						1	
14	74	38														0	0	0						0	
15	79	46														0	0	1						0	
16	78	42														0	0	2						0	
17	81	39														0	0	0						0	
18	84	46														0	0	0						1	
19	75	47														0	D	0							B
20	77	47														0		0	♂					0	0
21	79	45														0		0						0	0
22	72	40														0		0						?	0
23	66	50														0		0							0
24	68	55														0		0							0
25	59	32														0		0							0
26	62	32														D		D							♂
Total			38	12	16	49	15	40	29	40	12	11	4	12	35	26	24	11	♂	10	8	4	13	28	♂
Average		19.8																							

[1] The "B" at the head of each column shows that the aphid was born on the date indicated.
[2] The "D" immediately following each column of figures shows that the aphid died on the date indicated.
The total number of young for each female is given at the foot of each column.

TABLE IV.—*Line of generations of Macrosiphum granarium at Richmond, Ind., in 1908*

Generation.	Date of birth.	Date of first young.	Age at birth of first young.	Date of last young.	Productive period.	Life after last young.	Number of young.	Average young per day of productive period.	Largest number of young in one day.	Date of death or disappearance.	Total length of life.
First-born generation series:			*Days.*		*Days.*	*Days.*					*Days.*
1	Mar. 27	Apr. 19	23	May 26	37	12	38	1.0+	4	June 9	74
2	Apr. 19	May 9	20	May 17	8	1	12	1.5	2	May 18	29
3	May 9	May 18	9	May 27	9	4	16	1.7+	4	May 31	22
4	May 18	May 27	9	June 25	29	2	49	1.6+	4	June 27	40
5	May 27	June 7	11	June 20	13	2	15	1.1+	2	June 22	26
6	June 7	June 15	8	July 5	20	3	40	2	4	July 8	31
7	June 15	June 23	8	July 4	11	1	29	2.6+	4	July 5	20
8	June 23	July 3	10	July 18	15	1	40	2.6+	5	July 20	27
9	July 3	July 11	8	July 19	8	1	12	1.5	5	...do	17
10	July 11	July 19	8	July 26	7	1	11	1.5+	2	July 27	16
11	July 19	July 29	10	July 29	1	5	4	4	4	Aug. 3	15
12	July 29	Aug. 6	8	Aug. 17	11	0	12	1.0+	4	Aug. 17	19
13	Aug. 6	Aug. 18	12	Sept. 9	22	0	35	1.5+	4	Sept. 9	34
14	Aug. 18	Aug. 30	12	Oct. 13	44	13	26	.5+	3	Oct. 26	69
15	Aug. 30	Sept. 11	12	Sept. 25	14	24	24	1.0+	5	Oct. 19	50
16	Sept. 11	Sept. 29	18	Oct. 17	19	9	11	.5+	2	Oct. 26	45
17	Sept. 29										
Last-born generation series:											
2	May 27	June 10	14	July 1	21	3	10	.4+	3	July 4	38
3	July 1	July 13	12	July 16	3	3	8	2.6+	4	July 19	18
4	July 16	Aug. 3	18	Aug. 6	3	2	4	1.3+	2	Aug. 8	23
5	Aug. 6	Aug. 23	17	Sept. 5	13	4	13	1	2	Sept. 9	34
6	Sept. 5	Sept. 13	8	Oct. 19	36	3	28	.7+	2	Oct. 22	48
7	Oct. 19										

The other generation series are not tabulated as there do not seem to be sufficiently striking differences to justify it. One generation series ran to 18 in the direct line of first born and to 8 in the last born. Other generation series ran below the one tabulated. One female in the series not tabulated began producing young at the age of 7 days; one female produced 52 young and another lived 79 days. The writer has complete records of the number of young produced by 117 females. They produced 2,333 young, or an average of 19.9+ young each. The average length of time from birth to the production of young for 91 individuals was 12.6+ days. The average productive period for 99 individuals was 16.7 days. The productive period for one female (not in the tabulated series) was 48 days. The average length of life for 89 individuals was 34.3 days.

COLOR VARIATION IN RELATION TO THE SEXES

All who are familiar with this species of Aphididae will probably recall having seen them clustered on heads of wheat just before harvest, and noted distinct variation in color. The majority of them at this time are strongly tinged with pink. There seems to be no satisfactory explanation for the occurrence of the pink forms at this time. The pink forms occur again in October, and this is the signal for the appearance of the

sexes. The writer has taken both pink and green individuals in the summer and kept the progeny of each isolated in rearing cages until fall. The sexes appeared among the descendants from the pink individuals, but very sparingly from the descendants of the green ones. This may not hold in every instance, but it has been the experience of the writer that the sexes can be obtained with certainty by starting with the pink summer forms.

If the cages are examined closely when the sexes begin to appear in the fall, two distinct types of adults will be noted. One is the usual green form (Pl. B, 2) and the other will have a pinkish tinge (Pl. B, 1). If the pink wingless individuals are isolated it will be found that they produce two kinds of young, one slightly tinged with pink (Pl. B, 4) and another a deep pink (Pl. B, 3). The color of the mother after she begins producing young is due in the main to the pinkish young showing through the body wall. The slightly pink individuals are usually produced first. The offspring of the pink wingless individuals usually all become winged, the deep-pink individuals developing to winged males, while the pale-pink winged individuals are viviparous and produce the wingless, yellow, oviparous females (Pl. B, 5). This fact was not known definitely by the writer until the fall of 1909, when he isolated a few pink wingless viviparous females in order to learn what sex their offspring would be. In every case the results were as just stated.

In the fall of 1910 pink wingless individuals were again isolated for observation on their progeny. The results obtained entirely corroborated the data of 1909. In the fall of 1911 a large series was isolated as in 1909 and 1910. A heavy storm accompanied by very low temperatures put an end to the observations before the data were complete, killing all individuals under observation, since the rearing cages offered little natural protection from cold.

Since 1909 and 1910 the writer has found that the winged viviparous females of this series [1] may produce only viviparous individuals in some cases or both oviparous and viviparous, or may produce only the oviparous females. The males are produced only by the wingless pink viviparous females and the oviparous females are produced only by the winged adults that develop from the slightly pinkish young. In other words, the males are sons of the pink viviparous females and the oviparous females are the granddaughters. In no case are oviparous females and males produced by the same mother. The oviparous females might be termed nieces of the males; they are never sisters of the males.

The following outline will illustrate more clearly the sequence of the sexes:

[1] The offspring of the pinkish wingless viviparous females of the autumn forms.

Outlines showing methods of sequence of the autumn forms of Macrosiphum granarium

First method:

- Parental type... → Forms of first generation offspring. → Forms of second generation offspring.
- Pinkish wingless viviparous females.
  - Pinkish nymphs, becoming winged viviparous females. → Yellow, wingless oviparous females only.
  - Deep-pink nymphs, becoming winged males only.

Second method:

- Parental type... → Forms of first generation offspring. → Forms of second generation offspring.
- Pinkish wingless viviparous females.
  - A. Pinkish nymphs, becoming winged viviparous females. →
    - a. Yellow, wingless oviparous females only;
    - or
    - b. Wingless viviparous females only;
    - or
    - c. Winged and wingless viviparous females;
    - or
    - d. Yellow, wingless oviparous females and winged and wingless viviparous females.
  - B. Pinkish nymphs, becoming wingless viviparous females. → Winged males and winged and wingless viviparous females.
  - C. Deep-pink nymphs, becoming winged males only.

It will be seen, therefore, that in a single cage there may be green, slightly pink, deep-pink, and pale-yellow individuals—quite a wide range in color for a single species.

INFLUENCE OF TEMPERATURE ON PRODUCTION OF SEXES

During the first week in October, 1912, pinkish forms were plentiful in the stock cages and the sexes had begun to appear sparingly. A large number of pinkish individuals that showed promise of producing the sexes were isolated and placed in one of the greenhouses of the Purdue Experiment Station to hasten the production of the sexes and that more data on the progeny of the pink wingless females that appear at this time might be gathered. The greenhouse was kept at a temperature between 50° and 70° F., and the writer thought that since the sexes had begun to appear their numbers could be rapidly increased by placing them in a warmer temperature. Almost the opposite effect was produced. A number of males but only a very few oviparous females appeared. The ones that were obtained were probably born just before or very soon after they were placed in the greenhouse. A stock cage that was left outdoors produced quite a number of oviparous females and

males. This is not conclusive proof, but it certainly indicates that temperatures below 50° F. for a daily minimum in some way exert an influence on the normal production of the sexes.

OCCURRENCE OF THE SEXES AND THE PROPORTION OF MALES TO FEMALES

The first published record on the sexes is by Sanderson (13), who secured them from indoor rearing cages in April. These were the progeny of individuals taken in the fields in January. Notes on file in the Bureau of Entomology show that the late F. M. Webster made observations on the sexes as early as 1884 at Oxford, Ind. He records the males appearing as early as September and females in October and November. Although the egg was observed, no record was made relative to the stem mothers.

These observations in regard to the sexes agree very well with observations made by the writer. The young males first made their appearance during the last week in September or the first week in October. The females usually appear a little later. The males are likely to appear any time during the winter if kept in breeding cages indoors. In rearing cages indoors they appeared sparingly from September to April, inclusive. No oviparous females occurred during the winter. Males undoubtedly outnumber the females from the very fact of their occurrence both in and out of season. During the breeding season (October and November), however, the oviparous females usually outnumber the males, as there are from a fourth to a half as many pink individuals (mothers of oviparous females) as there are males. Each of the slightly pink females may produce from 6 to 20 oviparous females, and that would bring the numbers of the oviparous females far ahead of those of the males.

MATING

Mating occurs sometimes during the first two or three days after the female becomes adult, and oviposition begins in ½ to 5 or 6 days, depending upon the temperature. Females refuse to deposit eggs before mating. If the male is not present the bodies of the females become almost twice the normal size. In one case 5 females were isolated from males for about 10 days or more. Their bodies increased greatly in size, but no eggs were deposited. At the end of that period males were placed in the cage. Mating soon took place. In 10 days there were 13 eggs in the cage but all were infertile.

AGE OF OVIPAROUS FEMALES AT OVIPOSITION

The length of time for maturity of oviparous as for viviparous females depends largely upon temperature. Under the same conditions the oviparous females develop in about the same length of time as the viviparous. Large numbers of both sexes never reach maturity because of

low temperature. One oviparous female became adult in 9 days indoors. Another, born the same day and kept outdoors, developed in 12 days. The age at oviposition, therefore, would be from 14 to 20 days, depending upon the temperature and the presence of the males. The eggs are deposited on the leaves of the plant and on the sides of the cage.

FECUNDITY OF OVIPAROUS FEMALES

The largest number of eggs produced by a single female is 18, the productive period lasting from November 2 to December 1 (1909). Complete records on 20 individuals give an average of 8.4 eggs. The duration of the productive period is from 8 to 29 days.

LENGTH OF LIFE OF THE SEXES

The majority of the oviparous females observed by the writer lived until killed by very low temperatures in November or December. The males do not live quite a month. The female just mentioned, that produced 18 eggs, lived over a month after oviposition began, and was then killed by a severe freeze. Add to this her developmental period and she would be at least 1½ months old. This would probably be high for an average.

MOLTING

Molting experiments have been conducted with each form and it was found that the stem mothers, winged and wingless forms, males, and oviparous females, without exception, molt four times.

NATURAL ENEMIES

APHIDIUS NIGRIPES

The most efficient enemy of *M. granarium* is undoubtedly *Aphidius nigripes* Ashmead. As soon as *A. nigripes* becomes abundant, the brown leather-like, almost circular bodies of the aphids will be noticed firmly attached to the plant. These contain the immature stage of the parasite. Just before harvest, if the infestation of *M. granarium* is heavy, the heads of grain will be almost covered with their brown, dead bodies.

In the fall of 1908 sufficient data were secured to establish the parthenogenetic habits of this parasite. It produces only males under these conditions.

On October 7, 1907, at Richmond, Ind., two virgin females were introduced into a cage with a number of *M. granarium* that had been grown in confinement and had not been parasitized previously. They began oviposition at once. They would approach the aphid cautiously, bend the abdomen under until the tip extended beyond the head, then quickly stab the aphid. There does not seem to be any favorite point of attack, the parasite thrusting at the nearest point. The cage was kept out of doors in the rearing shelter and on November 1 the aphids began to

turn brown. On November 4 they had the usual leather-like appearance and each one was firmly glued to the leaf. On November 23 part of these old bodies were taken indoors and kept at the ordinary room temperature. The temperature went much lower at night than during the day, as the fire was allowed to go out. On December 2, male *A. nigripes* began to emerge and on the 4th all had emerged that were brought indoors. The ones that were left outside were still in the larval stage. This is probably the stage in which they pass the winter.

OTHER INSECT ENEMIES

The late F. M. Webster made more observations on the parasites and predacious enemies of *M. granarium* than any other entomologist. His notes from 1884 to 1890 that are on file in the Bureau of Entomology record the following insects as attacking this aphid:

COLEOPTERA

Podabrus tomentosus Say.
Coccinella 9-notata Herbst.
Hippodamia parenthesis Say.
H. convergens Guérin.
H. 13-punctata Linnaeus.
H. glacialis Fabricius.
Anatis 15-punctata Olivier.
Megilla maculata De Geer.

DIPTERA

Allograpta obliqua Say.
Sphaerophoria cylindrica Say.
Xanthogramma emarginata Say.

HYMENOPTERA

Aphidius avenaphis Fitch.
(*Dioeretus*) *Praon americanus* Ashmead.
(*D.*) *Praon brunneiventris* Ashmead (= *Praon americanus*).
(*D.*) *Praon ferruginipes* Ashmead (= *Praon americanus*).
Isocratus vulgaris Walker.
Encyrtus websteri Howard.
Pachyneuron micans Howard.
Allotria tritici Fitch.

Riley (9) records in addition the following:

COLEOPTERA

Coccinella sanguinea Linnaeus.

DIPTERA

Syrphus americanus Wiedemann.

HYMENOPTERA

Aphidius granariaphis Cook.
Tetrastichus ingratus Howard [*nomen nudum*]
Megaspilus niger Curtis.

All of the parasites listed, however, are not primary. In recent years two species, *Pachyneuron* sp. and *Allotria* sp., have been definitely proved to be secondary parasites. It is very probable that others in the list will be proved secondary upon further study.

FUNGUS ENEMIES

This aphid seems to be very susceptible to fungus attack. During warm, moist weather rearing cages have to be carefully watched or fungus will soon gain control. It undoubtedly destroys many aphids in the fields also.

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PLATE B

Forms of *Macrosiphum granarium:*

1.—Mother of males and grandmother of oviparous females.
2.—Typical green viviparous female.
3.—Pupa of male.
4.—Pupa of the mother of oviparous females.
5.—Oviparous female.

Henry Fox, artist.

A Hoen & Co Baltimore

PLATE 33

Macrosiphum granarium:

A.—Winged viviparous female: *a*, cornicle.
B.—Winged male.

A

B

A
B
C
D
E
F

PLATE 34

Macrosiphum granarium:

A.—Antenna of male.
B.—Antenna of winged viviparous female.
C.—Hind tibia of oviparous female.
D.—Antenna of wingless viviparous female.
E.—Antenna of wingless oviparous female.
F.—Antenna of stem mother

A SPECIFIC MOSAIC DISEASE IN NICOTIANA VISCOSUM DISTINCT FROM THE MOSAIC DISEASE OF TOBACCO

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During the summer of 1915 many plants of *Nicotiana viscosum* and first-generation plants of the cross *N. tabacum* ♀ × *N. viscosum* ♂ were grown in the field at Arlington, Va. Late in the season three plants of *N. viscosum* and one of the hybrid plants showed unmistakable symptoms of a typical mosaic disease. From the fact that the species viscosum and its hybrids had never before shown symptoms of disease from inoculations made with the virus of the ordinary mosaic disease of tobacco, these affected plants were taken into the greenhouse for further study. It has now been established that this mosaic disease affecting *N. viscosum* and its hybrids is biologically very different from the ordinary form of mosaic disease affecting varieties of *N. tabacum*, tomatoes (*Lycopersicon esculentum*), etc. Ordinary tobacco and also tomatoes appear to be quite immune from the type of mosaic disease in *N. viscosum*. Experiments have shown that this mosaic disease is infectious to plants of *N. viscosum*, although it appears that the disease is not as readily transferred by needle inoculations as the ordinary form of the mosaic disease, and longer periods of time are usually required before the disease comes into evidence.

A number of distinct varieties of *N. tabacum* have been crossed with *N. viscosum*, including Maryland Mammoth, White Burley, and Connecticut Broadleaf. In these crosses the pollen of *N. viscosum* has been transferred to the pistils of *N. tabacum*. In size, general appearance, and habit of growth first-generation plants of these crosses resemble much more closely the female parent (*N. tabacum*) than the male parent (*N. viscosum*). In general appearance the leaves and blossoms also resemble very closely the leaves and blossoms of the female parent. These first-generation plants inherit more strongly the visible physical characteristics of the female parent. They possess, however, certain physiological characteristics peculiar to the male parent (*N. viscosum*). This is indicated by the fact that they, like *N. viscosum*, appear to be immune to that form of mosaic disease which affects varieties of *N. tabacum*, but are susceptible to the mosaic disease affecting *N. viscosum*. The disease is readily obtained in these hybrids by grafting upon them scions taken from plants of *N. viscosum*. It is much more difficult to obtain the disease by needle inoculations. All phases of catacorolla in the blossoms and mottling and distortions in the leaves are shown in these hybrids affected with the

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mosaic disease of *N. viscosum* as in ordinary tobacco plants affected with the common form of the mosaic disease (Pls. 35 and 36.) The mosaic disease of *N. viscosum* produces more or less mottling and distortion in the blossoms of these plants. The abnormality known as catacorolla, however, has never appeared in connection with the disease.

TABLE I.—*Inoculations made with the expressed sap of scions of N. viscosum grafted upon ordinary tobacco (N. tabacum)*

Number of plants inoculated (Connecticut Broadleaf).	Date of inoculations.	Material used.	Symptoms of mosaic disease in scion of *N. viscosum.*	Results.
	1915.			
10	Dec. 16	Sap of scion of *N. viscosum* grafted on mosaic stock of *N. tabacum* several weeks.	None.....	All healthy.
10	16	.....do.....	...do.....	3 mosaic.
10	16	Tap water (control).....	.....	All healthy.
	1916.			
10	Jan. 5	Sap of scion of *N. viscosum* grafted upon mosaic stock of *N. tabacum* till scion was in bloom.	None.....	7 mosaic.
10	5	Sap of stock upon which above scion was grafted (symptoms in stock severe).	.....	10 mosaic.
10	5	Sap of scion of *N. viscosum* grafted upon mosaic stock of *N. tabacum* till scion was in bloom.	None.....	2 mosaic.
10	5	Tap water (control).....	.....	All healthy.
10	8	Sap of scion of *N. viscosum* grafted on stock of *N. tabacum* several weeks.	None.....	Do.
10	8	Tap water (control).....	.....	Do.
10	12	Sap of scion of *N. viscosum* grafted several weeks on stock of *N. tabacum.*	None.....	Do.
10	12	.....do.....	...do.....	Do.
10	12	.....do.....	...do.....	Do.
10	29	Sap of scion of *N. viscosum* grafted upon stock of *N. tabacum* till scion was in bloom.	...do.....	Do.
10	29	.....do.....	...do.....	Do.
10	29	.....do.....	...do.....	Do.
10	29	.....do.....	...do.....	Do.
10	29	.....do.....	...do.....	Do.
10	29	Sap of scion of *N. viscosum* grafted upon stock of *N. tabacum* several weeks.	...do.....	Do.
10	29	Tap water and healthy sap.....	.....	Do.

Although the species *N. viscosum* is susceptible to a mosaic disease peculiar to itself, this species of Nicotiana appears to be immune to the ordinary form of mosaic disease affecting *N. tabacum*. Likewise, first-generation plants of the cross *N. tabacum* ♀ × *N. viscosum* ♂ appear to be quite as immune from the disease as the species *N. viscosum*. All

methods of inoculation which have been attempted with these plants have been without success. It has been shown that the virus was not present in these plants by extracting the sap of all parts of the plants and testing its infectivity by making inoculations into young tobacco plants. These inoculations have never produced infection. Furthermore, many successful grafts have been made between *N. tabacum* and *N. viscosum*, using *N. tabacum* as the stock. As soon as the *N. viscosum* scion had started to grow, the stock (*N. tabacum*) was inoculated with the ordinary form of the mosaic disease. Scions of *N. viscosum* in many instances remained upon the mosaic stocks for many weeks and finally blossomed, yet symptoms of the mosaic disease never appeared in the blossoms or leaves. In all instances inoculation tests have been made to determine if the infective principle of the disease was present in the sap of the immune scions. As shown in Table I, these scions in many instances appeared to be entirely free from infection. In other instances the sap proved to be more or less infectious to tobacco plants. Why the sap of the scion should carry the infective principle at one time and not at another can not at present be explained.

The mosaic disease affecting *N. viscosum* appears to be identical in all its symptoms with the mosaic disease of tobacco (*N. tabacum*). The virus of the disease, however, has behaved very differently from the virus of the mosaic disease of tobacco in all inoculation tests. With the exception of *Datura fastuosa* (Golden Queen variety), and *Datura stramonium*, no other plants of the solanaceous family have been found susceptible to the virus of the mosaic disease affecting *N. viscosum*. Although peppers and tomatoes are very susceptible to the virus of the mosaic disease of tobacco, these plants appear to be immune from the virus of the mosaic disease affecting *N. viscosum*, or at least highly resistant to it, since the most persistent and rigorous needle inoculations have failed to produce infection. The most rigorous methods of inoculation have also failed to produce either the mosaic disease of tobacco or the mosaic disease of *N. viscosum* in the Irish potato (*Solanum tuberosum*).

Datura stramonium is the only solanaceous plant which has given evidence of being susceptible to both mosaic diseases. Inoculations made at different times with different lots of virus producing the mosaic disease in *N. tabacum* have given very different results. In some tests the plants were highly resistant to infection. In other tests similar methods of inoculation gave a high percentage of mosaic-diseased plants. It has not been determined whether this variability indicates differences in the infective properties of the virus or differences in the relative resistance of different lots of plants. In one experiment 18 young vigorous plants of *Datura stramonium* were divided into two lots of 9 plants each. One lot was inoculated at many points in the stems and leaves with the virus of the mosaic disease of *N. viscosum*. The remain-

ing 9 plants were inoculated in the same manner with the virus of the mosaic disease of *N. tabacum*. For a period of several weeks numerous inoculations were made from time to time in each lot of plants. The plants of each lot were also cut back severely several times and the virus inoculated into all cut surfaces. The plants were kept under observation for several months. Every plant in the series inoculated with the virus of the mosaic disease of *N. viscosum* developed the disease, the first observable symptoms appearing 21 days after the first inoculation. In this experiment the datura plants proved to be highly resistant to the virus of the mosaic disease of *N. tabacum*, as none became diseased. In those plants affected with the mosaic disease of *N. viscosum* the symptoms were particularly malignant. The leaves became greatly curled, wrinkled, and depauperate. Mottling of the leaves, however, was less marked than in those instances where *Datura stramonium* has been affected with the mosaic disease of *N. tabacum*.

The virus of the mosaic disease affecting *N. viscosum* differs from the virus of the mosaic disease of tobacco as follows:

CHARACTERISTICS OF THE VIRUS OF THE MOSAIC DISEASE OF TOBACCO (N. TABACUM)	CHARACTERISTICS OF THE VIRUS OF THE MOSAIC DISEASE AFFECTING N. VISCOSUM
(1) Transmission through the seed has never occurred.	(1) Transmission through the seed has never occurred.
(2) Incubation period short (minimum 6 days).	(2) Incubation period in *N. viscosum* rather long (minimum may be several weeks).
(3) Needle inoculations readily produce the disease.	(3) Needle inoculation rather uncertain. Grafts of mosaic-diseased shoots of *N. viscosum* upon susceptible plants readily produce infection.
(4) All attempts to infect belladonna (*Atropa belladonna*) and *Solanum tuberosum* have been unsuccessful.	(4) All attempts to infect belladonna and *Solanum tuberosum* have been unsuccessful.
(5) All attempts to infect pokeweed (*Phytolacca decandra*) have been unsuccessful.	(5) All attempts to infect pokeweed have been unsuccessful.
(6) All attempts to infect the hybrid *N. tabacum* ♀ × *N. viscosum* ♂ have been unsuccessful.	(6) The hybrid *N. tabacum* ♀ × *N. viscosum* ♂ is susceptible, manifesting typical symptoms of the disease.
(7) Highly infectious to tomatoes.	(7) All attempts to infect tomatoes have been unsuccessful.
(8) Infectious to the pepper (*Capsicum cerasiforme*).	(8) All attempts to inoculate the pepper have been unsuccessful.
(9) All attempts to infect sweet peas have been unsuccessful.	(9) All attempts to infect sweet peas have been unsuccessful.
(10) All attempts to infect *Datura fastuosa* (Golden Queen variety) have been unsuccessful.	(10) *Datura fastuosa* (Golden Queen variety) is susceptible, manifesting symptoms more or less typical of the disease.
(11) Affects Jimson weed (*Datura stramonium*) producing typical symptoms.	(11) Affects Jimson weed, producing symptoms very similar to those produced

Jimson weed, however, sometimes shows considerable resistance to the mosaic disease affecting *N. tabacum*.	by the ordinary mosaic disease of tobacco.
(12) Highly infectious and particularly malignant to *N. rustica*.	(12) All attempts to infect *N. rustica* have been unsuccessful.

The writer is of the opinion that this distinctive type of mosaic disease affecting *N. viscosum* has in some manner originated from the ordinary form of mosaic disease, possibly through the agency of insect transmission in the field. This does not seem improbable, since practically every susceptible plant in a half-acre field of ordinary tobacco in which the *N. viscosum* plants were grown became mosaic; and throughout the season both species were infested with great numbers of flea beetles. It is possible that insects may become efficient transmitters of disease where ordinary methods of artificial inoculation fail.

During the same season the writer's attention was called to the occurrence of typical symptoms of the mosaic disease in peppers grown in a field near by. To all outward appearances the plants were affected with a severe mosaic disease which gradually spread over the field and persisted in all affected plants. Tomato plants in adjoining rows, however, were unaffected. The expressed sap from the most severely attacked pepper plants failed to produce the mosaic disease in young tobacco plants (*N. tabacum*). Whether this mosaic disease was infectious to healthy pepper plants or might have been in any way related to the mosaic disease affecting *N. viscosum* was not determined.

In this connection it is interesting to note that various European investigators have reported that they were unable to inoculate other species of solanaceous plants with the virus of the mosaic disease of tobacco with which they worked. Thus, Mayer [1] failed to produce the disease in other solanaceous plants.

Iwanowski [2] has stated that the mosaic disease of tobacco does not occur upon *Datura stramonium* or *Hyoscyamus niger*.

Iwanowski,[3] in a later publication, stated that he had never known *Nicotiana rustica* to be affected by the mosaic disease.

Koning [4] also failed to communicate the mosaic disease of tobacco to *Datura stramonium*, *Hyoscyamus niger*, *Solanum tuberosum*, and *Petunia nyctaginifolia*.

Westerdijk,[5] working with a mosaic disease which was infectious to tomatoes, reported that she could not communicate this disease to

[1] Mayer, Adolf. Ueber die Mosaikkrankheit des Tabaks. *In* Landw. Vers. Stat., Bd. 32, p. 450–467, pl. 3. 1886.

[2] Iwanowski, D. Über die Mosaikkrankheit der Tabakspflanze. *In* Bul. Acad. Imp. Sci. St. Petersb., n. s. v. 3 (v. 35), no. 1, p. 67–70. 1892.

[3] Iwanowski, D. Über die Mosaikkrankheit der Tabakspflanze. *In* Centbl. Bakt. [etc.] Abt. 2, Bd. 5, No. 8, p. 250–254, 2 fig. 1899.

[4] Koning, C. J. Der Tabak . . . p. 71–86, fig. 13–15. Amsterdam, 1900.

[5] Westerdijk, Johanna. Die Mosaikkrankheit der Tomaten. 19 p., 3. pl. Amsterdam, 1910. [Meded. Phytopath. Lab. "Wille Commelin Scholten." Amsterdam.]

tobacco. Likewise, she could not infect tomato plants with the sap of a mosaic disease of tobacco with which she worked.

The constancy of these negative results is rather striking. It is possible that the type of mosaic disease with which European investigators worked may not have been quite so readily communicable to plants of other species and genera of the solanaceous family as the type in the writer's possession. It has been more or less generally believed in Europe that *N. rustica* was even immune to the mosaic disease affecting tobacco. In the writer's experience the virus of the common form of the mosaic disease is not only very infectious but particularly malignant to plants of *N. rustica*. Likewise, the disease is readily communicable to all the more distinct varieties of tomatoes, petunia, *Datura stramonium*, and is highly infectious to *Hyoscyamus niger*.

PLATE 35

Leaves of *Nicotiana viscosum* affected with the mosaic disease. This mosaic disease does not affect ordinary tobacco (*N. tabacum*); nor does the mosaic disease affecting ordinary tobacco affect *N. viscosum*.

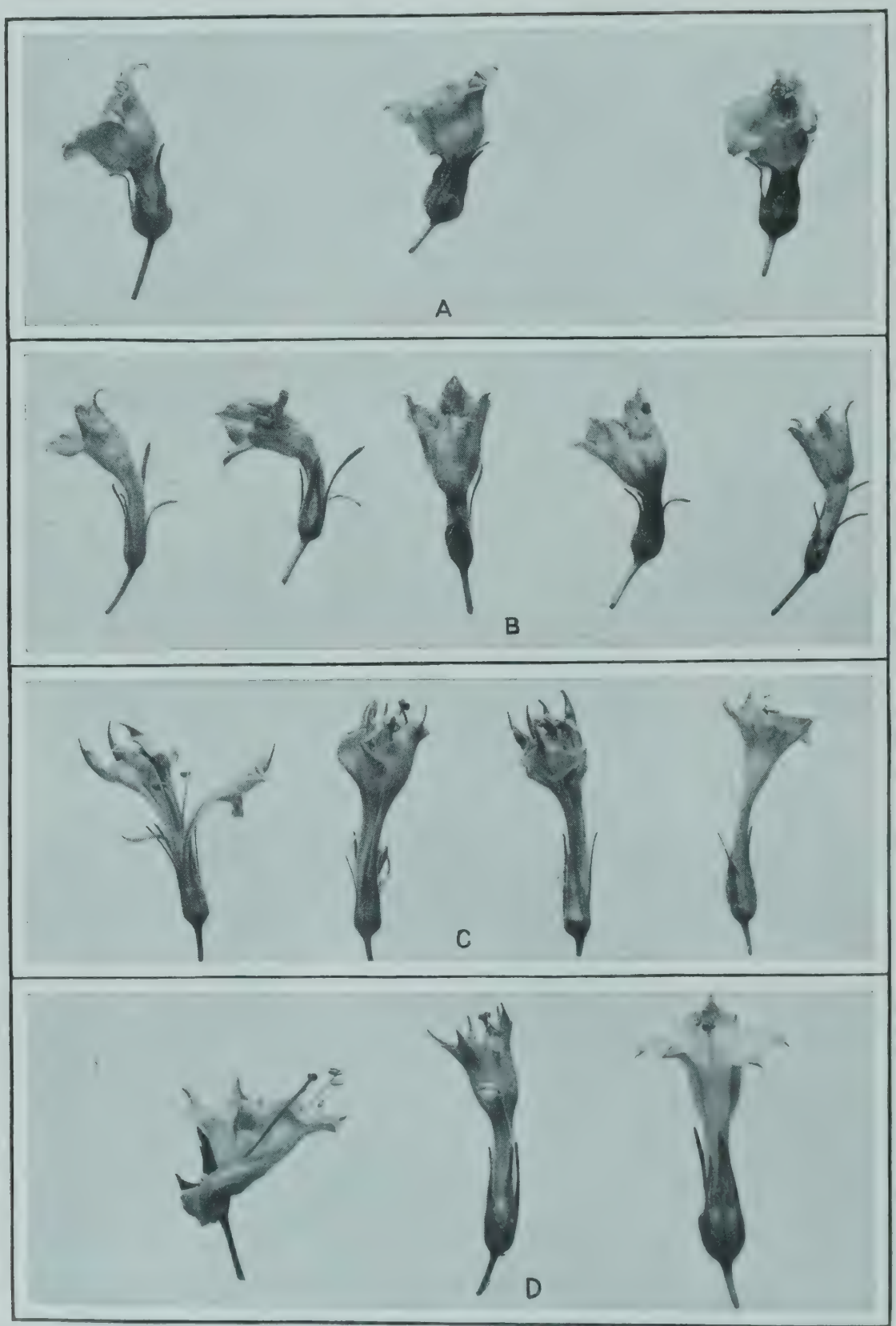
A
B
C
D

PLATE 36

A.—Normal blossoms from healthy plants of *Nicotiana viscosum.*

B.—Depauperate blossoms from mosaic plants affected with the mosaic disease peculiar to *N. viscosum.* This disease is distinct from the ordinary form of the mosaic disease affecting varieties of *N. tabacum* and does not affect them.

C, D.—Blossoms showing catacorolla, etc., as a result of the mosaic disease affecting *Nicotiana viscosum.* These are from first-generation plants of the cross Connecticut Broadleaf tobacco ♀ × *N. viscosum* ♂. This hybrid appears to be immune from the ordinary mosaic disease affecting the female parent, but is susceptible to the mosaic disease affecting the male parent, *N. viscosum.* Although this mosaic disease has never produced instances of catacorolla in *N. viscosum*, all phases of catacorolla are produced in the hybrid. Catacorolla is a common malformation in varieties of *N. tabacum* as a result of the ordinary form of the mosaic disease.

SYNTOMASPIS DRUPARUM, THE APPLE-SEED CHALCID

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INTRODUCTION

Since the publication by Crosby (7 [1], p. 369) of his paper on the apple-seed chalcid (*Syntomaspis druparum* Boh.) this insect has attracted more and more attention among those associated with the apple industry, and numerous letters relating to it have been received at the Bureau of Entomology. The frequency and wide distribution of these inquiries and complaints seemed to warrant a rather detailed investigation of the insect, and the writer has spent portions of the past two seasons (1914 and 1915) in such an investigation. The biological work was done at the field laboratory for the investigation of deciduous-fruit insects of the Bureau of Entomology at North East, Pa., while the field observations have been conducted throughout the northern tier of States from Vermont to Michigan.

DESCRIPTION OF THE ADULT INSECT

The adult insect is somewhat wasplike in appearance, bright green, with coppery or bronzy metallic reflections, brownish yellow legs, and clear hyalin wings. The female (Pl. 37, A) is normally about 4 mm. in length and is provided with a slender ovipositor slightly longer than the body. The male (Pl. 37, B) is somewhat smaller than the female.

DISTRIBUTION IN THE UNITED STATES

The apple-seed chalcid apparently occurs throughout the northern tier of States, at least from Vermont to Michigan. It has not been found in Ohio or Indiana. At the time of the writer's visit to those States there was a very small crop of apples, and none especially suitable for the attack of the insect were found. But in the same season the chalcid was found in the seeds of a wild seedling at Benton Harbor, Mich. The writer has also found it as far south as Clearfield, Pa., and some years earlier what was almost undoubtedly the larva of this species was found in a crab apple (*Malus* sp.) at Vienna, Va. It is probably distributed throughout the eastern part of the country wherever small seedling apples (*Malus sylvestris*) are to be found.

HISTORICAL REVIEW

Crosby (7, p. 369; 9) has given a nearly complete résumé of the history of the apple-seed chalcid in Europe, where it has been well treated

[1] Reference is made by number to "Literature cited," p. 501.

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recently by Mokrzecki (4). His own papers record the only original observations on the species in America. The insect was first discovered by Prof. Crosby in July, 1906, at Ithaca, N. Y., when he found the seeds of crab apples to contain the partly grown larvæ. His first report of his discovery appeared in 1908 (6, p. 38), and the following year he published his full account (7, p. 369). In the latter paper he summarizes most of the previously published accounts of the species and records in detail his own observations in regard to life history, habits, distribution, and host fruits and gives descriptions of the stages. His 1912 paper (9) consists of further résumés of European literature.

From the wide distribution of the species it is evident that it must have been present though undiscovered in America for a long time, but any statement as to the time of its introduction can be nothing more than speculation. However, that there have been many opportunities for its introduction in the past and that it has been repeatedly introduced in fruit from Europe can not be doubted. It may even have been brought to America before its discovery in Europe, and its establishment here may have been effected at that early time; for it is a historical fact that in the early days of American history apples were imported and their seeds planted by the colonists. Much of the early spread of the apple to the West was due to the Indians, who planted in favorable spots the seeds from apples given them by the settlers. These trees, planted mostly along the trails to the West, would form easy avenues of distribution, and it is quite likely that they and their progeny have aided in the spread of the insect.

EFFECT UPON FRUIT

The only externally visible effect of infestation is caused by the oviposition puncture, which, after a few days, appears as a minute scar situated in a small, shallow dimple. From this scar to the seed extends a discolored line. Under ordinary circumstances of growth and infestation the fruit apparently is able to outgrow both of these manifestations of injury. But occasionally, especially when fruit is scarce or the insects very abundant, the gross injury due to repeated puncturing at nearly the same spot causes permanent and deep dimpling, together with corky, discolored streaks in the flesh. However, even in 1915, when suitable fruit was rather scarce, the season cold, and the chalcids abundant in the region of North East, Pa., such injury was the exception rather than the rule; and in 1914, when the converse of these conditions prevailed, no single case of severe distortion that could be attributed to this species was found. Distorted fruit is shown in Plate 38, A, B, C.

Frequently injury caused by other insects is attributed to the apple-seed chalcid because at the time the injury is noted this species is the only one present. As an example of this, the case of an orchard near Clearfield, Pa., may be cited. The bulk of the fruit in this

orchard in 1914 was very badly distorted, and specimens sent to various entomologists were pronounced to be the work of *Syntomaspis druparum* for the reason that larvæ of this species were found in the seed. The writer visited this orchard in October and examined large numbers of the fruits, but found the chalcid larvæ in comparatively few. Obviously the chalcid was not responsible for such extensive injury, especially in view of the fact that the fruit of wild seedlings almost within the boundaries of the orchard was heavily infested by the chalcid and showed no sign of distortion. Observation in the same orchard the following spring disclosed the fact that it was grossly infested by both species of apple red bugs (*Lygidea mendax* Reut. and *Heterocordylus malinus* Reut.), which had come from *Crataegus* sp. and wild crab in the surrounding woods. These were the insects responsible for the injury to the apples, and the chalcids were able to infest the seeds because of the stunting due to the red-bug injury. It should be stated, in justification of this mistaken determination, that both of the insects concerned are of comparatively recent discovery, and their work is familiar to but few entomologists.

When first infested, the seeds show the laceration caused by the ovipositor surrounded by a brownish area; but as they darken, the injured area heals and ultimately appears as a lighter area, a repeatedly punctured seed having a mottled appearance. At full growth infested seeds are less plump and more irregular than normal seeds. Infested and sound seeds are shown in Plate 39.

Crosby (7, p. 369) states that in the Lady apple the texture of the flesh is considerably injured. This has not been apparent to the writer, for on visiting an orchard containing trees of this variety, from which the owner had picked what he termed an "unusually fine crop," fully two-thirds of the apples examined were heavily infested by the chalcid; but it was impossible to tell whether an apple was infested without examining the seeds or making an almost microscopic examination of the skin for the minute oviposition scars. Special attention was paid to apples of commercial size and color, and a very large percentage was found infested. Moreover, fruit of this variety has been purchased on the Washington market 50 per cent of the seeds of which contained larvæ of the chalcid.

Horvath records failure in Budapest of apple seed to produce a good stand on account of infestation by the chalcid.

VARIETIES AND SPECIES OF FRUIT ATTACKED

The apple-seed chalcid has been found to infest a great variety of fruits. The original description (1, p. 361–362) was based on specimens reared from the seeds of *Sorbus scandica*. The species was redescribed by Thomson (2, p. 76) from seeds of *Sorbus* sp. Rodzianko (5, p. 593–602) reared it from *Sorbus aria*, *Pyrus baccata*, and *Malus sylvestris*. In

Europe it has been mentioned a number of times in connection with the apple, but frequently without any statement as to the nature of the fruit. Porchinsky (3) records the rearing of a species of Torymus from the seeds of wild pear (*Pyrus communis*), but gives no specific determination of the insect. It may have been *Syntomaspis druparum*, but not certainly so. Crosby (7, p. 369) lists the Lady apple, natural fruit, the wild crab (*Pyrus* [*Malus*] *coronaria*), and the following cultivated crab apples: *Pyrus* [*Malus*] *sibricia* var. *striata*, *Pyrus* [*Malus*] *floribunda*, *Pyrus* [*Malus*] *prunifoliae*, and *Pyrus* [*Malus*] *ioensis*. He also states that larvæ, apparently the same, were found in the seeds of *Sorbus latifolia*, but that the adults were not reared. In correspondence with the Bureau of Entomology Mr. M. L. Benn, of Coudersport, Pa., states that he has found infestation by the seed chalcid in Northern Spy, Baldwin, Fameuse, Wagener, Russet, Tolman Sweet, and two seedlings. Mr. G. McL. Stevens, of Orwell, Vt., reported it as attacking Lady apples at Orwell, Vt., and natural fruit at Peru, N. Y., while Mr. A. E. Stene reports it from Kingston, R. I., in the seeds of crab apple.

The writer's observations on the species began a number of years ago at Vienna, Va., where what was undoubtedly the larva of the seed chalcid was found in a seed of a crab apple.

Since the beginning of the work on the species, many varieties of apples have been examined under many conditions and in widely separated localities. At practically every point visited nearly every variety of natural fruit, except the largest, has been found to be more or less generally infested.

Among cultivated varieties the Lady apple only is apparently subject to very serious attack, this variety being frequently very heavily infested. The ordinary commercial varieties are never infested except in neglected and run-down orchards or when fruit is stunted by the overloading of trees or by the attack of some other insect or disease. The reason for the immunity of the ordinary apples of commerce from attack is purely mechanical, in that, at the time the chalcids are ovipositing, such fruit is so large that the ovipositor will not reach to the seeds. However, under the circumstances enumerated above, such varieties are occasionally more or less infested, though never very heavily so. Larvæ have been found by the writer in neglected orchards at North East, Pa., in the following varieties: French Russet, Northern Spy, and Baldwin. In a large orchard near Clearfield, Pa., which in 1914 was very badly infested by red bugs (*Lygidea mendax* Reut. and *Heterocordylus malinus* Reut.), and the fruit much distorted and stunted thereby, only Grimes Golden, Ben Davis, and Missouri of the many varieties examined were infested. Of these Grimes Golden showed about 25 per cent of the fruit infested, from one to four seeds in the infested apples containing larvæ of the chalcid. Of the two other varieties only one apple each was found to be infested.

Crab apples, both cultivated and wild, are very frequently infested, but invariably to a less extent than the small wild seedlings of the true apple, and it is evident that the latter is the natural host of the insect. On one occasion the opportunity offered to compare the infestation in these two classes of fruit where wild crabs and wild seedlings were found growing side by side. Only about 50 per cent of the crabs were infested, and rarely more than one seed to the fruit contained larvæ, while the infestation in the seedling apples was practically 100 per cent, uninfested seeds being scarce.

Although the fruit of the common mountain ash (*Sorbus americana*) has been repeatedly and extensively examined, the writer has never found any trace of infestation by this or any other chalcid.

Neither pears nor the fruit of *Crataegus* sp. exposed to the attack of the chalcid in cages were infested, although attempts at oviposition on the latter were repeatedly observed and many fruits were exposed to attack and later examined.

LIFE HISTORY OF THE CHALCID

The life-history data given below were obtained very largely by propagation of the apple-seed chalcid on wild seedling fruit at North East, Pa., and involved the examination of many hundreds of apples. The female insects were caged on fruit for one day in cages constructed of mica lamp chimneys and cheesecloth (Pl. 40, D).

EMERGENCE IN SPRING

The insects reared in 1914 were from a lot of apples that had been kept in Washington, D. C., during the previous winter and until about May 15, when they were shipped to North East, Pa. The earlier spring of Washington undoubtedly hastened somewhat the emergence of some of the earlier reared adults, for they began to emerge from the seeds on May 26, which was some time before the apples at North East were at the proper stage for oviposition. However, they did not begin to appear in numbers until after the middle of June, the heaviest emergence occurring during the week of June 22–29 and the last on July 5. The adults reared in 1915 were from apples that passed the winter in an unprotected wire cage at North East. The first to emerge appeared on June 16 and the last on July 16, with the heaviest emergence, as in 1914, during about the last week of June.

There appears to be very little, if any, difference in the time of emergence of the sexes. In the more normal emergence of 1915 a few males appeared before any females, and the few belated individuals that emerged after the first few days of July were all females. But during every other day of the emergence season some individuals of each sex appeared.

RELATIVE ABUNDANCE OF SEXES

The rearings during 1914 consisted of 254 females and 85 males, 74.9 and 25.1 per cent, respectively. In 1915, 316 females and 100 males were reared, 75.9 and 24.1 per cent, respectively. These figures show a ratio of about 3 females to each male.

OVIPOSITION

AGE AT BEGINNING.—The female chalcids become mature and able to deposit eggs within a very short time after emergence, for they have been repeatedly observed in the act of oviposition within two days after issuing from the seed.

AGE OF FRUIT.—At the time of the heaviest emergence of the chalcids apples have grown, depending on the variety, to a diameter of from a half inch to somewhat over an inch. The seeds have attained nearly full growth, but have not begun to harden. Most of the space within the seed is occupied by a jelly-like mass, with the small embryo at one end. Between this and the outer seed coat is a rather thick mucilaginous layer.

METHOD AND TIME REQUIRED.—In ovipositing, the female chalcid first feels carefully over the surface of the apple with her antennæ; then, when she has located a place to her liking, she raises the abdomen, at the same time releasing the ovipositor from its sheath and lowering it until its tip is against the surface of the apple directly beneath the posterior end of the thorax. The abdomen is now perpendicular to its normal axis. With pressure accompanied by a slow swinging of the abdomen from side to side the ovipositor is forced slowly into the apple until inserted to its full length. At the end of this time the abdomen has resumed nearly its normal position except that the hypopygidium is directed downward with the ovipositor, making a triangular projection below the abdomen. Now the ovipositor is several times partially withdrawn and thrust back until the insect is apparently satisfied that it has been properly inserted, when she remains perfectly quiet for a considerable period, during which the egg is deposited. When this is finished the ovipositor is withdrawn and swung back into its sheath. The whole process occupies, on the average, somewhat in excess of five minutes. Living chalcids in various phases of the act of oviposition are shown in Plate 40, B, C, while in A one is shown attempting oviposition in the fruit of *Crataegus* sp.

POINT OF ATTACK.—When oviposition first begins, most of the punctures are made around the middle of the apple, but later in the season the attack is shifted nearer to the calyx end. This is apparently made necessary by the fact that the growth of the apples makes it impossible for the ovipositor to reach the seed from the side. Figure 1 shows the position of punctures in fruit and seed.

RELATION BETWEEN PUNCTURES MADE AND EGGS DEPOSITED.—That the instinct of the ovipositing female in locating the seed is not so strong and unerring as might at first be supposed, when the frequent very high percentage of infestation of the seeds is considered, is indicated by the number of punctures made in the seed compared with the number showing on the surface of the apple. The puncture is much more conspicuous on the white seed than on the skin of the apple; yet one fruit that had been punctured 36 times had only five punctures on its seeds. It is unlikely that each puncture made represents an egg deposited, but rather that many punctures represent unsuccessful attempts at finding seed. This is borne out by the observations on ovipositing females, which frequently inserted their ovipositors repeatedly at almost the same point before ultimately going through all the phases of the act of oviposition. It is not even probable that every puncture in the seed represents the deposition of an egg. No definite assertion on this point can be made, since the eggs are rather difficult to find.

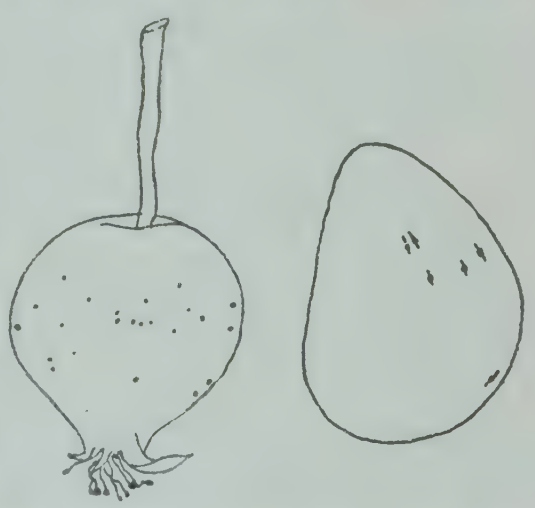

FIG. 1.—*Syntomaspis druparum:* Apple, natural size, and seed, enlarged, showing oviposition punctures. (Original.)

PLACE OF DEPOSITION OF EGG.—Apparently it is the aim of the insect to place its egg in the central gelatinous mass of the seed, and from the position of many of the punctures it is impossible that through them the ovipositor could have reached this body. Many punctures are on the side of the seed, in such position that the ovipositor must have been nearly tangent to the surface of the seed.

Sometimes eggs are deposited in the mucilaginous layer next to the seed coat, but the resulting larvæ apparently never mature, for many dead larvæ of the first instar have been found in this situation, and living larvæ found there have always been in the first instar and far behind, in growth, the larvæ of the same age in the more favorable jelly-like body.

OVIPOSITION PERIOD.—The longest period during which any of the caged females were ovipositing in 1914 was from June 25 to July 21, a period of 26 days, two insects in the same cage having died on the same date. During this time 48 apples were exposed, and all were more or less infested. Others lived for periods ranging from 3 to 24 days, the quicker deaths being due apparently to the sun striking the cages.

EGG

DESCRIPTION.—The egg (fig. 2) is elongate oval, roundly pointed at the caudal end, and prolonged at the cephalic end into a slender, twisted pedicle about one-fourth the diameter and nearly as long as the body

of the egg. Exclusive of this appendage, the egg is about 0.55 mm. in length by about a fourth as thick in the middle. It is yellowish white and without sculpture. Within two days after oviposition the embryo can be seen to have drawn away from the poles, and in some the cephalic constriction can be seen.

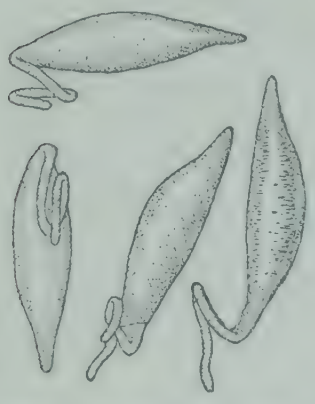

FIG. 2.—*Syntomaspis druparum:* Eggs. Highly magnified. (Original.)

INCUBATION PERIOD.—In 1914 the eggs began to hatch on the sixth day after deposition, and by the eighth day all had hatched. In 1915 hatching commenced on the seventh day and continued until the tenth day.

LARVA

NUMBER AND DESCRIPTION OF INSTARS.—The newly hatched larva (fig. 3) is about 0.4 mm. in length by about a fourth as thick at the thickest point, which is at the junction of the thoracic and abdominal segments. From this point it tapers in both directions, but is much smaller at the caudal end. The body, including the head, consists of 14 segments; the 3 thoracic segments are about equal in length, and the abdominal segments gradually decrease in length toward the caudal end. The head is nearly hemispherical and rather heavily chitinized. The mouth opening is nearly circular and surrounded by a raised rim. Owing to the minute size and delicacy of the mouth parts, except the strong mandibles, it is difficult to determine definitely their exact relation to each other, but they appear to be about as in the illustration (fig. 4). The mandibles are long, strongly curved, and dark colored. They cross in the middle of the mouth opening. The head is from 0.108 to 0.123 mm. in breadth and the mandibles 0.021 mm. in length. At full growth the larva of the first instar is slightly less than 1 mm. in length.

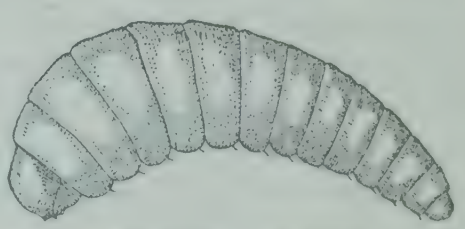

FIG. 3.—*Syntomaspis druparum:* Newly hatched larva. Highly magnified. (Original.)

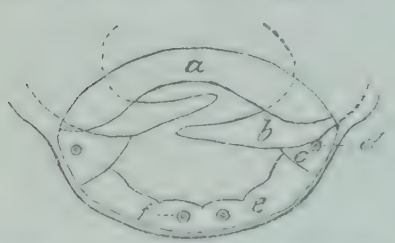

FIG. 4.—*Syntomaspis druparum:* Mouth parts of larva of first instar *a*, Labrum; *b*, mandible; *c*, maxilla; *d*, maxillary palpus; *e*, labium; *f*, labial palpus. Highly magnified. (Original.)

The larva of the second instar is very similar in general appearance to that of the first instar, having the same tapering form though being somewhat stouter. It can, however, be easily distinguished by the weaker chitinization of the oral region and the change in the form of the mandibles, which at this molt assume a form more similar to those of the full-grown larva. The brown color of the mandibles is confined to their tips, and they are very stout at the base and much less strongly curved (fig. 5, *a*). The head in this instar is from 0.184 to 0.215 mm. broad,

and the mandibles from 0.036 to 0.039 mm. long. At full growth the second instar is about 1.5 mm. long.

With each succeeding molt the larva becomes gradually stouter and less tapering behind until at full growth it is more than a third as thick as long, with the caudal end but little more tapering than the head end, and the head becomes relatively smaller and more retracted within the thorax. With each molt the head and mandibles increase markedly in size, and the latter change somewhat in form. These changes in measurements and form constitute the only real differentiating characters until in the last instar the spiracles become open and visible.

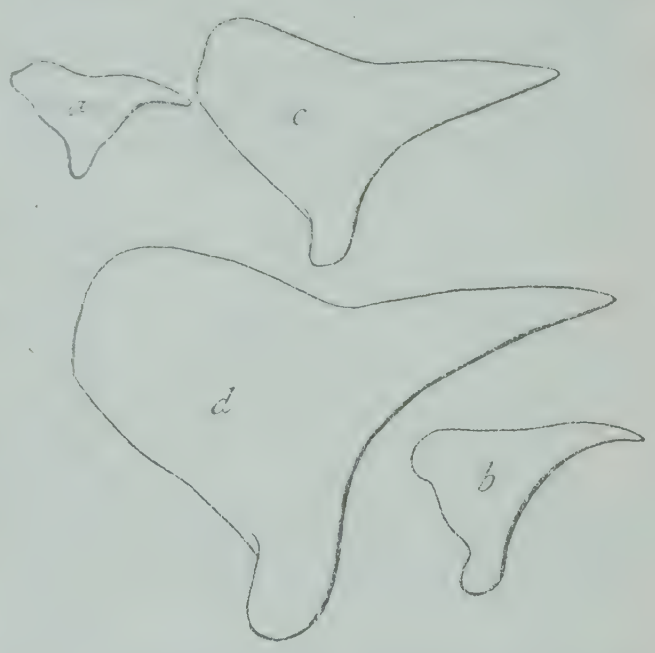

FIG. 5.—*Syntomaspis druparum:* Mandibles of larvæ of various instars. *a*, Second; *b*, third; *c*, fourth; *d*, fifth. Highly magnified. (Original.)

The head of the third-instar larva varies in breadth from 0.277 to 0.308 mm. and the length of the mandibles from 0.050 to 0.057 mm. The latter (fig. 5, *b*) are curved toward the apex. In the fourth instar the head is from 0.415 to 0.461 mm. broad and the mandibles (fig. 5, *c*) 0.079 to 0.086 mm. long and nearly straight at the apex.

The full-grown or fifth-instar larva (fig. 6) is of the typical chalcid form, rather spindle-shaped but somewhat curved toward the ventral side, with the head short and flattened and partially retracted within the first thoracic segment. The mesothoracic and metathoracic segments and the first seven segments of the abdomen bears each a pair of minute spiracles. Fully fed larvæ are mostly from 4.5 to 5 mm. long, but a few, which develop in small seeds, are much smaller, the smallest measured being 3 mm. in length. The head is from 0.554 to 0.6 mm. broad and the mandible (fig. 5, *d*), the blade of which is rather slender and nearly straight, is from 0.111 to 0.129 mm. long. The arrangement of the mouth parts is shown in figure 7.

FIG. 6.—*Syntomaspis druparum.* Full-grown larva. Much enlarged. (Original.)

PLACE OF FEEDING.—The earlier feeding of the larva is done in the gelatinous portion of the developing seed. In the meantime the embryo is developing and the gelatinous body is being absorbed. Before the

latter entirely disappears the larva begins to feed on the cotyledons, eating out a pit on one edge or one side and ultimately devouring the last traces of the embryo.

DURATION OF INSTARS AND FEEDING PERIOD.—The two seasons during which observations on the life history of the apple-seed chalcid were made were quite different, and the development of the larvæ was consequently quite different in point of time required. The summer of 1915 was unusually cold, and the larvæ required about a week longer to complete their development than those of 1914.

The observations of 1914 were complicated and rendered somewhat difficult of interpretation because of an unexpected infestation from natural sources. The presence of the species in the locality was not discovered until too late to escape the infestation from that source. However, the natural infestation was, as a rule, either considerably earlier or considerably later than that in the cages, and some information of value can be secured from the data obtained.

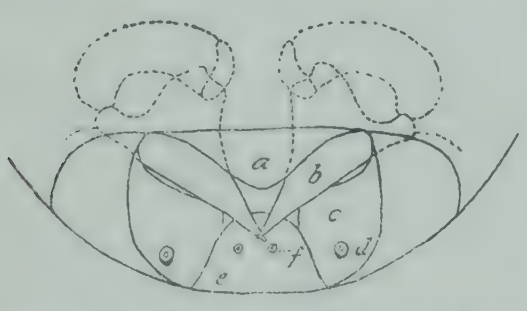

FIG. 7.—*Syntomaspis druparum:* Mouth parts of full-grown larva. *a*, Labrum; *b*, mandible; *c*, maxilla; *d*, maxillary palpus; *e*, labium; *f*, labial palpus. Highly magnified. (Original.)

In 1915 these difficulties were eliminated by the expedient of bagging all fruit to be used in the work both before and after it was exposed to the attack of the insects in the cages.

In 1914 the first individual of each instar was found about four days after the first of the immediately preceding instar, all transformations from one instar to the next taking place within a period of about a week. In about 45 days from oviposition practically all the larvæ had finished feeding. Table I gives the data on the development of the seed chalcid during 1914. From this table are excluded all individuals the presence of which was obviously due to natural infestation.

TABLE I.—*Development of Syntomaspis druparum at North East, Pa., in 1914*

Period from infestation to examination.	Date of infestation.	Date of examination.	Stages of insect found.				
			Eggs.	First larval.	Second larval.	Third larval.	Fourth larval.	Fifth larval.
Days.								
3–4	June 20–21	June 24	6					
4–5	July 3–4	July 8	3					
5–6	June 18–19	June 24	4					
6	June 25	July 1	17	2				
	July 2	July 8	3	3				
	July 9	July 15	2	4				
7	July 1	July 1	1	3				
8	June 23	do		6				
	July 7	July 15		12				
8–9	June 29–30	July 8		3				
9	July 6	July 15		3				
	July 7	July 16		4				

TABLE I.—*Development of Syntomapsis druparum at North East, Pa., in 1914*—Contd.

Period from infestation to examination.	Date of infestation.	Date of examination.	Stages of insect found.					
			Eggs.	First larval.	Second larval.	Third larval.	Fourth larval.	Fifth larval.
Days.								
8–10	July 12–14	July 22		1				
10	July 6	July 16		1				
10–11	June 18–19	June 29		17				
	June 27–28	July 8		8				
11	July 5	July 16		2	4			
12	June 26	July 8		8				
12–13	July 3–4	July 16		1	3			
13	June 24	July 8		12	5			
	July 9	July 22			3			
14	June 24	July 8		3	5			
	July 2	July 16			2	1		
	July 8	July 22			3	2		
15	June 23	July 8			4			
16	June 22	...do			1	3		
17	July 5	July 22				9		
17–18	June 20–21	July 8			1	7		
18	June 23	July 11				3	15	
18–19	June 27–28	July 16				4	4	
	July 3–4	July 22				2	3	
19	June 22	July 11				3	12	
20	June 26	July 16				2	3	
	July 2	July 22					7	
22	June 24	July 16					4	1
23	June 23	...do					6	2
24	June 22	...do					6	4
24–25	June 27–28	July 22					2	1
25–26	June 20–21	July 16					7	10
26	June 26	July 22						4
27	June 25	...do						6
29	June 23	...do						6
31	June 22	July 23						4
32–33	June 20–21	...do						4
38–39	.....do	July 29						2
40–41	June 18–19	...do						1

In 1915 the earliest hatching took place on the seventh day after oviposition, the earliest first molt on the sixteenth day, the earliest second molt on the twenty-first day, the earliest third molt on the twenty-fifth day, and the earliest last molt on the twenty-ninth day, and the first larva to consume the entire seed contents had done so on the forty-ninth day. The last larva to finish feeding required 57 days.

Table II shows all the life-history data obtained during 1915 from cage-infested apples. As will be noted, all apples used in this work were infested during a period of 5 days from June 28 to July 2. Thus all individuals were developing under practically identical conditions. It will also be noted that many of the belated first-instar larvæ and eggs were found in the mucilaginous tissue surrounding the central gelatinous body.

TABLE II.—*Development of Syntomaspis druparum at North East, Pa., in 1915*

Period from oviposition to examination.	Date of oviposition.	Date of examination.	Stage and number of individuals found.						Remarks.
			Egg.	First larval.	Second larval.	Third larval.	Fourth larval.	Fifth larval.	
Days.									
6	June 29	July 5	10	.....	.....	.....	.....	.....	
7	June 28	...do.....	.....	3	.....	.....	.....	.....	
7	July 2	July 9	1	.....	.....	.....	.....	.....	
8	July 1	...do.....	6	6	.....	.....	.....	.....	
8	July 2	July 10	1	3	.....	.....	.....	.....	
9	June 28	July 7	1	5	.....	.....	.....	.....	
10	...do.....	July 8	.....	8	.....	.....	.....	.....	
11	...do.....	July 9	.....	7	.....	.....	.....	.....	
12	...do.....	July 10	.....	5	.....	.....	.....	.....	
12	July 2	July 14	.....	11	.....	.....	.....	.....	
13	June 28	July 11	.....	6	.....	.....	.....	.....	
13	July 1	July 14	.....	10	.....	.....	.....	.....	
14	June 28	July 12	.....	2	.....	.....	.....	.....	
15	...do.....	July 13	.....	25	.....	.....	.....	.....	
16	...do.....	July 14	.....	12	2	.....	.....	.....	
17	...do.....	July 15	.....	4	5	.....	.....	.....	
18	...do.....	July 16	.....	3	5	.....	.....	.....	
19	...do.....	July 17	1	6	10	.....	.....	.....	Egg and two first-stage larvæ were in mucilaginous tissue at large end of seed.
19	July 1	July 20	.....	2	3	.....	.....	.....	
20	June 28	July 18	.....	4	5	.....	.....	.....	
21	...do.....	July 19	.....	1	5	2	.....	.....	First-stage larva in mucilaginous tissue.
22	...do.....	July 20	1	1	5	2	.....	.....	Egg and first-stage larva in mucilaginous tissue, seeds considerably hardened.
22	June 29	July 21	.....	1	4	8	.....	.....	First-stage larvæ in mucilaginous tissue.
23	June 28	...do.....	.....	3	.....	6	.....	.....	Do.
24	...do.....	July 22	.....	.....	.....	2	.....	.....	
24	June 29	July 23	.....	.....	2	3	.....	.....	
25	June 28	...do.....	.....	.....	.....	7	2	.....	
26	...do.....	July 24	.....	.....	2	3	3	.....	
27	...do.....	July 25	.....	.....	1	1	6	.....	
28	...do.....	July 26	.....	.....	.....	.....	8	.....	
29	...do.....	July 27	.....	.....	.....	.....	6	.....	
29	July 2	July 31	.....	.....	.....	3	15	4	
30	June 28	July 28	.....	.....	.....	.....	4	.....	
30	July 1	July 31	.....	.....	.....	.....	7	1	
30	July 2	Aug. 1	.....	.....	.....	.....	4	4	
31	July 1	...do.....	.....	.....	.....	.....	8	7	
32	June 29	July 31	.....	1	.....	.....	6	8	First-stage larva in mucilaginous tissue.
32	July 2	Aug. 3	.....	.....	.....	.....	2	7	
33	July 1	...do.....	.....	.....	.....	.....	1	10	
33	July 2	Aug. 4	.....	.....	.....	.....	1	7	
34	July 1	...do.....	.....	.....	.....	.....	3	13	
35	...do.....	Aug. 5	.....	.....	.....	.....	1	13	
36	...do.....	Aug. 6	.....	.....	.....	.....	.....	8	
37	...do.....	Aug. 7	.....	.....	.....	.....	2	9	
38	July 2	Aug. 9	.....	.....	.....	.....	.....	13	
39	July 1	...do.....	.....	.....	.....	.....	1	8	
39	July 2	Aug. 10	.....	.....	.....	.....	3	20	
40	July 1	...do.....	.....	.....	.....	.....	.....	4	
40	July 2	Aug. 11	.....	.....	.....	.....	.....	17	
41	...do.....	Aug. 12	.....	.....	.....	.....	.....	12	
42	June 29	Aug. 10	.....	.....	.....	.....	.....	8	
43	June 28	...do.....	.....	.....	.....	.....	.....	2	
43	June 29	Aug. 11	.....	.....	.....	.....	.....	15	
44	...do.....	Aug. 12	.....	.....	.....	.....	.....	12	
45	...do.....	Aug. 13	.....	.....	.....	.....	1	10	
46	...do.....	Aug. 14	.....	.....	.....	.....	.....	8	
47	...do.....	Aug. 15	.....	.....	.....	.....	.....	11	
48	...do.....	Aug. 16	.....	.....	.....	.....	.....	7	
49	...do.....	Aug. 17	.....	.....	.....	.....	.....	9	2 had consumed entire seed contents.
50	July 2	Aug. 21	.....	.....	.....	.....	.....	9	
51	...do.....	Aug. 22	.....	.....	.....	.....	.....	7	3 had consumed entire seed contents.
52	...do.....	Aug. 23	.....	.....	.....	.....	.....	10	Do.
53	...do.....	Aug. 24	.....	.....	.....	.....	.....	8	4 had consumed entire seed contents.
54	...do.....	Aug. 25	.....	.....	.....	.....	.....	7	5 had consumed entire seed contents.
55	...do.....	Aug. 26	.....	.....	.....	.....	.....	7	Do.
56	...do.....	Aug. 27	.....	.....	.....	.....	.....	9	7 had consumed entire seed contents.
57	...do.....	Aug. 28	.....	.....	.....	.....	.....	7	Do.

On August 30, 1915, two days after the last cage-infested apple had been examined, 165 seeds infested naturally were examined to determine whether all larvæ had by that time finished feeding. Of the larvæ found, 132, or exactly 80 per cent, had consumed the entire contents of the seed and the rest had practically done so. Of 50 larvæ examined on September 2, all had finished feeding. In other words, by the last of August all the larvæ had reached full growth.

NUMBER MATURING IN A SINGLE SEED.—In removing larvæ from apple seeds the fact has been observed that as they increase in size and age the likelihood of finding more than one in a seed decreases. It is not at all uncommon to find 6 or 7 very young larvæ in a single seed, even in an apple naturally infested; but on only one occasion has more than 1 of the fifth instar been found within a single seed. In this case there were 2. The number is usually reduced to 1 before the fourth instar is reached. This reduction in number is brought about by the actual killing and eating of the surplus larvæ by the one which ultimately matures. On a number of occasions this cannibalistic habit has been observed, the larvæ concerned being usually in the second or third instar.

HIBERNATING LARVA.—When the larva has consumed its total supply of food it very shortly assumes what may be called the hibernating form (Pl. 38, D). This does not involve a molting of the skin but consists merely in longitudinal contraction of the body, the head and caudal segments being drawn in and the body becoming relatively thicker and more deeply wrinkled. In this condition it remains until the following spring.

BIENNIAL BROOD.—Not all of the larvæ from eggs of a given season finish their development and emerge as adults the following spring, but a large percentage of them remain as larvæ within the seeds until the second spring. This was suspected during the summer of 1914, when, on July 23, the writer, in examining some seeds infested in 1913, found some that still contained living larvæ. One hundred seeds were selected at random to determine roughly what percentage of the larvæ were likely to live over until the next spring. Of these 100 seeds, 54 contained dead larvæ, 26 living larvæ, and from 14 the adult insects had emerged. Of the living insects 65 per cent had not emerged. This lot of seed was kept until the summer of 1915 and count kept of the emerging adults. A total of 416 insects were reared in the second spring as against 339 in 1914. In other words, 55.1 per cent of the insects lived over two winters as larvæ.

It would appear that this curious habit serves to prevent extermination of the species by a season of no fruit.

PUPATION.—The larvæ begin to pupate during the latter half of May, the latest pupation, judging from the emergence of the adults, probably taking place from three weeks to a month later.

PUPA

DESCRIPTION.—The pupa (fig. 8) is normally, depending on the sex, from 3 to 4 mm. long, females being the larger. It is at first white, but later those parts that are chitinized in the adult become first brownish and later dark greenish; this color being really on the body of the adult, developing within the pupal skin and showing through the latter. The legs, wing pads, antennæ, and palpi are folded along the sides and venter, and in the female the ovipositor extends over the back, reaching nearly to the head.

FIG. 8.—*Syntomaspis druparum:* Pupa of female. Much enlarged. (Original.)

PUPAL PERIOD.—The pupal period is of about four weeks' duration, some ndividuals requiring slightly less and some slightly more than this period.

ECONOMIC IMPORTANCE

As has been pointed out on an earlier page, the only commercial fruits that are, under conditions of ordinary care, at all heavily infested by the seed chalcid are the Lady apple and, occasionally, crab apples, both varieties with very limited markets. Also, under normal conditions of growth distortion of fruit to such extent as to render it unmarketable is rather rare, and infestation by the chalcid apparently has no effect on the color of fruit. As pointed out by Crosby and as proven by the observations of the writer, Lady apples are apparently practically immune to the distortion of oviposition. These things being true, it is apparent that economically the seed chalcid is of little importance.

CONTROL OF THE CHALCID

NATURAL CONTROL.—Apparently the apple-seed chalcid has no specific enemies. No records of such are to be found in European or American literature, and none has come under the observation of the writer. Other apple insects, such as the codling moth, which sometimes devour the seeds, undoubtedly destroy a limited number of chalcid larvæ, and others, which feed in the fallen apples, account for the death of a few more. Some adult chalcids doubtless fall prey to birds, spiders, and other predators. But all of these together constitute only a very small measure of control.

Mortality among the hibernating larvæ is apparently very small also; for of 115 larvæ found in apples that had lain under the tree all through the winter of 1914–15 only three were dead, and each of these was in a seed that had been eaten into by some other insect. The mortality in seeds that become separated from the pulp may be higher, but as it is almost impossible to find such seeds no data on the point are available.

ARTIFICIAL CONTROL.—Inasmuch as the seed chalcid attacks normally only varieties that are grown on a very small scale it can be controlled with comparatively little effort by purely mechanical means. In the first place, all wild seedling apples and wild crab apples in the neighborhood of such varieties should be destroyed. This would not only eliminate the outside source of this insect, but also of many other much more serious pests. This should be done in the spring or summer, preferably in August after oviposition has ceased, to insure the destruction of the chalcid larvæ of the season. From the seed crop of the previous season there will still be the chalcids of the biennial brood for the next following season to contend with, but in two years this source of infestation will be entirely eliminated. In addition to the foregoing the careful destruction of all drop fruit and culls for two seasons will practically exterminate the chalcids. If the waste fruit is converted into cider, the pomace should be destroyed.

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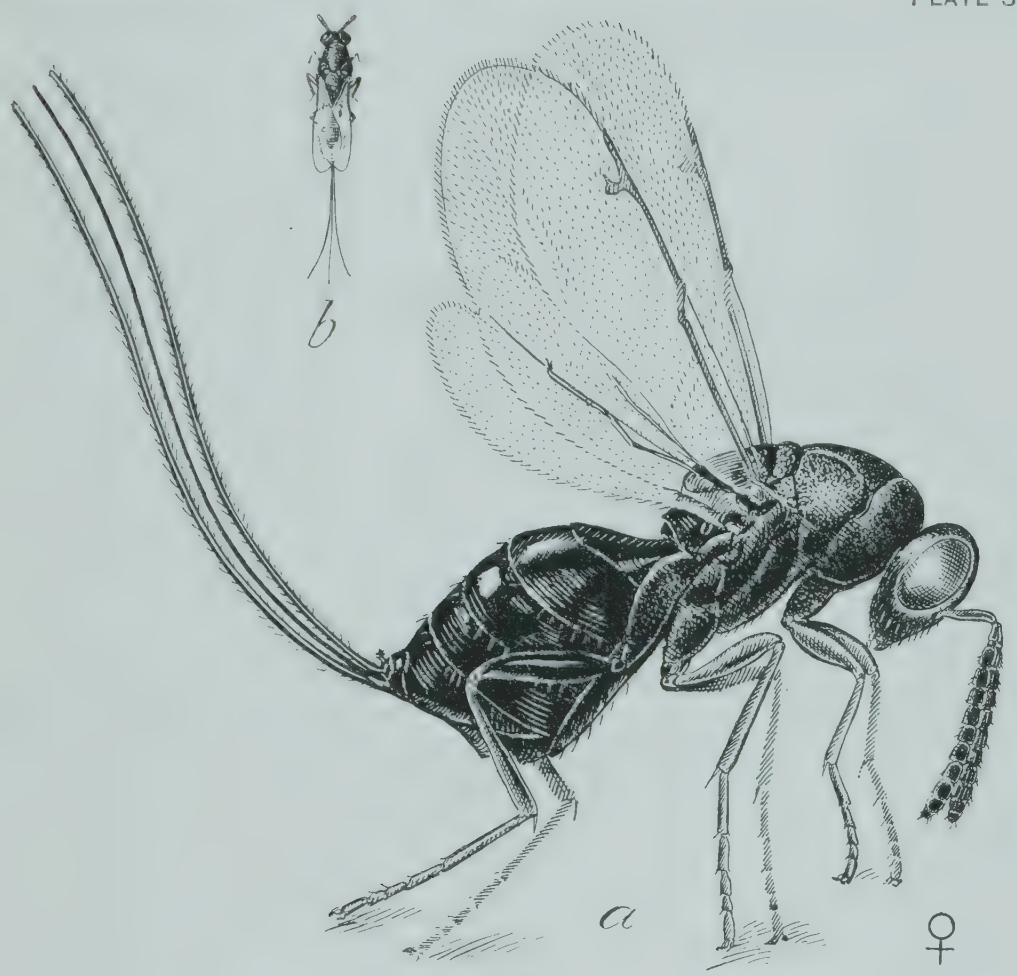
PLATE 37

Syntomaspis druparum:

A.—Adult female. *a*, greatly enlarged; *b*, × 3. (Original.)

B.—Adult male; outline of abdomen, lateral view, at right. Greatly enlarged. (Original.)



A

B

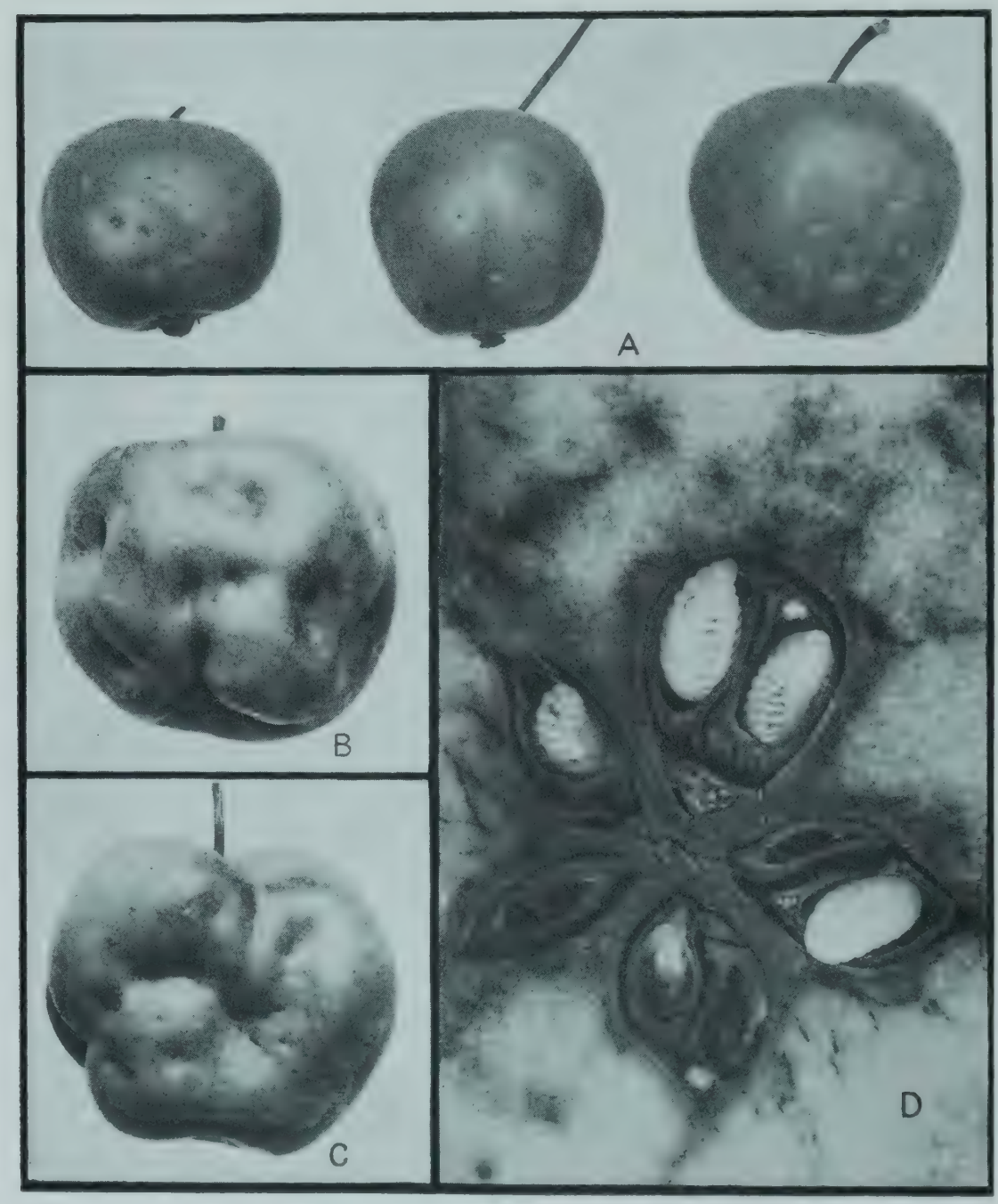

A
B
C
D

PLATE 38

Syntomaspis druparum: Apple injury and hibernating larvæ

A.—Usual type of injury resulting from oviposition. Natural size.
B, C.—Extreme type of injury resulting from oviposition. Natural size.
D.—Hibernating larvæ within seeds of an apple. Greatly enlarged.

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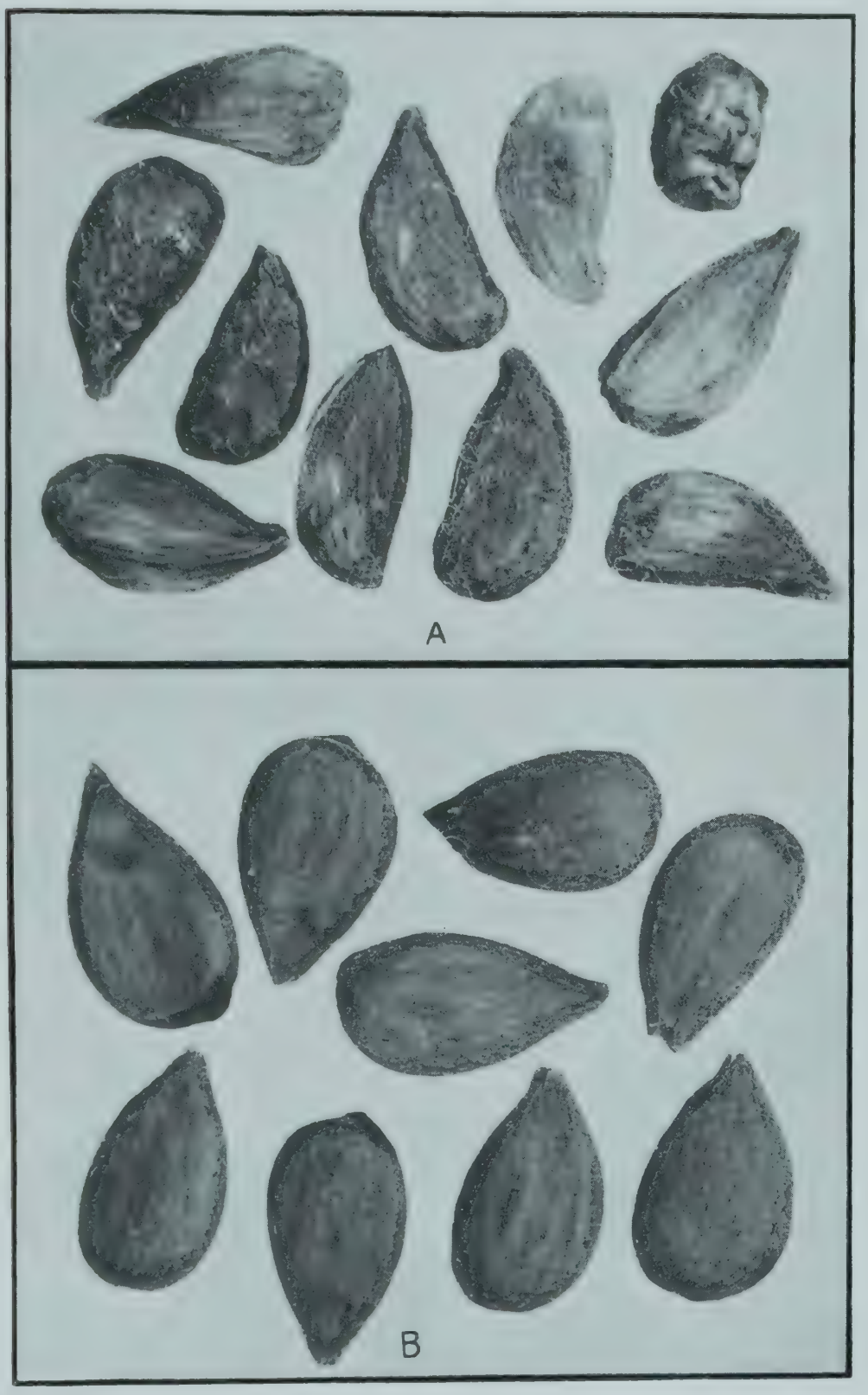
PLATE 39

Syntomaspis druparum: Infested and sound seeds of apples

A.—Infested seeds. Much enlarged.
B.—Sound seeds. Much enlarged.


A
B

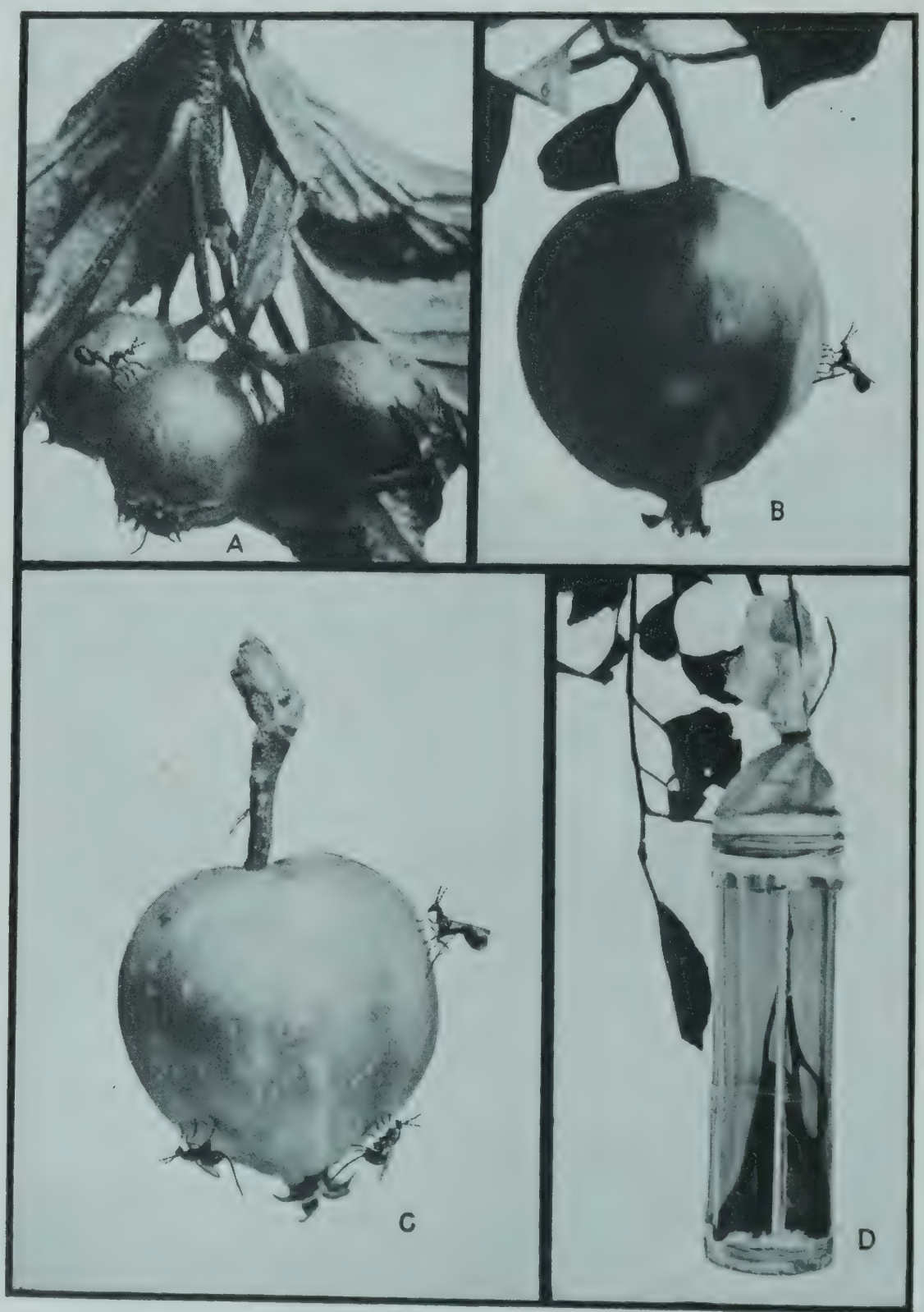

A
B
C
D

PLATE 40

Syntomaspis druparum: Oviposition

A.—Female ovipositing in fruit of *Crataegus* sp. Photographed from life. × 2.
B, C.—Oviposition in apples. Photographed from life. × 2.
D.—Mica cage used in the life-history studies of *Syntomaspis druparum*. × ⅓.

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ASSIMILATION OF IRON BY RICE FROM CERTAIN NUTRIENT SOLUTIONS

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INTRODUCTION

It has long been recognized that on calcareous soils certain plants do not make a normal growth and are often affected by chlorosis. This has been variously ascribed to the physical condition of the soil; an increasing assimilation of lime by the plant; a diminished assimilation of potash, iron, or phosphoric acid; or a diminished assimilation of all mineral nutrients due to the neutralization of acid root excretions. As already noted, work at this station with pineapples and upland rice indicated an insufficient assimilation of iron as the principal cause (4, 5)[1]

As calcareous soils have a slightly alkaline reaction it is important to know whether plants intolerant of calcareous soils are sensitive to an acid or alkaline reaction *per se*, and whether the reaction of the soil has any effect on the assimilation of iron. Various experiments with upland rice (*Oryza sativa*) in nutrient solutions and soil cultures have been conducted to gain information on this subject. The experiments with nutrient solutions reported here show the effect of the quantity and form of iron and reaction of the nutrient medium on the asssimilation of iron by rice.

PLAN AND EXPERIMENTAL METHODS

The plan of the work was to measure the growth of rice in acid, neutral, and alkaline nutrient solutions when supplied with 0.002 and 0.008 gm. of iron (Fe) per liter from ferrous sulphate, ferric chlorid, dialyzed iron, ferric citrate, and ferric tartrate; to determine the amount of iron taken up by the plants from these solutions; and to determine the amount of soluble iron actually present in the solutions at different times. A few other experiments were conducted to explain certain results obtained. Three preliminary experiments were also conducted before the general plan and exact detail of the experiments were decided upon.

[1] Reference is made by number to "Literature cited," p. 528.

Upland rice seedlings germinated over distilled water were placed in the nutrient solutions when the plumules were about 2 inches long. Water distilled from a cast-iron still with a block-tin condenser and stored in a tin-lined copper tank was used in the nutrient solutions. The plants were grown for 40 days in Erlenmeyer flasks of Jena or Nonsol glass. Flasks of 200 c. c. capacity were used for the first 25 days of each experiment and flasks of 500 c. c. capacity for the remaining 15 days. Transpired water was replaced with distilled water daily, and the plants were changed to fresh solutions every 4 days, except they were left in the solution 6 days before the first change. The plants were kept in a wire inclosure (five meshes to the inch) during fair weather and in a glass house during rains.

The nutrient solutions used were of the following compositions:

Acid solution.	*Gm.*	Neutral solution.	*Gm.*
Potassium nitrate (KNO_3)	10. 71	Potassium nitrate (KNO_3)	10. 71
Monobasic potassium phosphate (KH_2PO_4)...	7. 14	Monobasic potassium phosphate (KH_2PO_4) ..	3. 57
Sodium nitrate ($NaNO_3$)..	21. 43	Dibasic potassium phosphate (K_2HPO_4).....	3. 57
Sodium sulphate (Na_2SO_4)	3. 15	Sodium nitrate ($NaNO_3$)..	21. 43
Calcium chlorid ($CaCl_2$)..	2. 0	Sodium sulphate (Na_2SO_4)	3. 15
Magnesium chlorid ($MgCl_2$).....	2. 0	Calcium chlorid ($CaCl_2$)..	2. 0
Sulphuric acid (H_2SO_4)...	0. 245	Magnesium chlorid ($MgCl_2$).....	2. 0
Distilled water.....	100, 000	Distilled water.....	100, 000

The solution alkaline with carbonate of lime was the same as the neutral solution except for the addition of 0.41 gm. of precipitated calcium carbonate per liter. In the various experiments different quantities and kinds of iron were added to these solutions.

It is possible that the above solutions are not ideal for rice, although plants equal in size to exceptionally large field plants were grown in them. Previous and subsequent work with these solutions showed that the growth of rice was not increased by increasing the quantity of calcium chlorid or magnesium chlorid, by doubling the quantities of dibasic potassium phosphate and monobasic potassium phosphate, or by doubling the quantities of potassium nitrate and sodium nitrate. The concentration of total salts in the solutions was lower than usual, but afforded sufficient nutrients because of the frequent changes. There is an advantage in using dilute solutions in that imperfect balancing of the salts (antagonistic effects of the ions) may produce no injury in low concentrations.

Before each change the nutrient solutions were made up fresh from a stock solution and the iron added 18 hours before the plants were inserted. After emptying out the old solution, the flasks and roots were rinsed once with a small quantity of distilled water. These details seem unimportant, but, as will be seen later, alteration in such apparently trivial details may affect the results.

In analyzing the plants for iron the substance was ashed over a low flame without the addition of calcium acetate and iron determined colorimetrically by the method of Stokes and Cain (14). In attempting to estimate the soluble iron in the nutrient solutions, the solutions were filtered, the filtrate concentrated when necessary, and iron determined colorimetrically by the above method. The ordinary colorimetric method with potassium sulphocyanate (KSCN) was used in some of the preliminary work, but all the determinations reported were made by the method of Stokes and Cain unless otherwise specified. Blanks were run for iron with the acid and materials employed.

PRELIMINARY EXPERIMENTS

Following are the data of three preliminary experiments conducted before a uniform method was adopted:

EXPERIMENT 1 (Source of iron: Ferrous sulphate. Plants grown in double flasks).—It was at first thought it would be advantageous to grow the plants with their roots equally divided between two flasks, one flask to contain the acid solution and the other the neutral. By adding iron only to the acid solution in one lot and only to the neutral solution in another lot it was thought that decisive results would be obtained on the effect of the reaction of the solution on the assimilation of iron, as the only difference between the two lots would be in the solution in which iron occurred. The results, however, did not bear out the assumption.

Two plants were grown in each twin flask (A and B). Four flasks were taken as a unit and the units triplicated for each treatment. All solutions containing iron contained the same quantity, but in this experiment the quantity of iron was not kept constant during the 40 days of growth, as it was thought that at first so much iron was added as to obscure the effect of the reaction on assimilation. During the first 10 days 0.008 gm. of iron per liter was used, during the second 10 days 0.004 gm. of iron, and during the last 20 days 0.002 gm. of iron. In Table I are given the weights of the plants grown in the different solutions, together with the percentages of nitrogen and iron in the dry substance.

The color of the plants during growth was as follows: At 10 days plants 1 to 12 were strongly chlorotic, plants 25 to 36 were chlorotic, plants 13 to 24 and 49 to 60 were green, and plants 37 to 48 were dark green; from the twentieth to the fortieth day plants 1 to 12 were strongly chlorotic and all others were about the same normal green.

From the weights of the stalks and leaves it appears that with the quantities of iron used the reaction of the solution had little effect on the assimilation of iron. It also seems that there is no advantage in growing the plants with their roots divided between two flasks, as one-half the roots are not able to absorb sufficient iron for the full needs of

the plants.[1] This is evident from a comparison of the weights of plants 13 to 24, with 37 to 48, and of plants 25 to 36 with 49 to 60.

TABLE I.—*Comparative weights of rice plants grown in double flasks with ferrous sulphate in acid and neutral solutions and percentage of nitrogen and iron*

Nutrient solution in flask—		Flasks No.	Green weight of stalks and leaves.	Oven-dry weight of stalks and leaves.	Oven-dry weight of roots.	Average oven-dry weight of stalks and leaves.	Average oven-dry weight of whole plant.	Iron (Fe_2O_3) in dry stalks and leaves.	Nitrogen (N) in dry stalks and leaves.
A.	B.								
			Gm.	*Gm*	*Gm.*	*Gm.*	*Gm.*	*Per ct.*	*Per ct.*
Neutral	Acid	1–4	1. 42	0. 26	0. 10	.....	.....	.....	.....
		5–8	1. 39	. 26	. 09	.....	.....	.....	.....
		9–12	1. 32	. 26	. 10	0. 26	0. 36	0. 040	3. 90
Do	Acid+iron	13–16	36. 10	4. 78	1. 29	.....	.....	.....	.....
		17–20	38. 55	5. 09	1. 39	.....	.....	.....	.....
		21–24	28. 85	3. 93	1. 10	4. 60	5. 86	. 038	3. 72
Neutral+iron.	Acid	25–28	29. 62	4. 10	1. 14	.....	.....	.....	.....
		29–32	33. 80	4. 78	1. 23	.....	.....	.....	.....
		33–36	31. 19	4. 27	1. 17	4. 38	5. 56	. 023	3. 90
Acid+iron	Acid+iron	37–40	48. 42	5. 99	1. 87	.....	.....	.....	.....
		41–44	48. 70	6. 22	1. 88	.....	.....	.....	.....
		45–48	51. 97	6. 61	1. 91	6. 27	8. 16	. 038	3. 70
Neutral+iron.	Neutral+iron.	49–52	49. 25	6. 34	1. 67	.....	.....	.....	.....
		53–56	53. 67	7. 00	1. 91	.....	.....	.....	.....
		57–60	50. 49	6. 48	1. 70	6. 61	8.37	. 022	3. 80

EXPERIMENT 2 (Source of iron: Ferrous sulphate. Three different quantities of iron in acid and neutral solutions).—Since it was thought that in experiment 1 so much iron was used as to obscure partially the effect of the reaction on the assimilation of iron, an experiment was conducted using different quantities of iron in acid and neutral solutions. As a check on the previous results, two lots received iron at the same rate as in experiment 1—that is, 0.008 gm. of iron per liter for the first 10 days, 0.004 gm. for the second 10 days, and 0.002 gm. for the last 20 days of the experiment. The other lots received either 0.008 or 0.002 gm. per liter during the whole 40 days. Ferrous sulphate was used as the source of iron. One plant was grown in each flask, six flasks were taken as a unit, and the units triplicated for each treatment. The weights of the plants with the percentages of nitrogen, phosphoric acid, and iron in the dry substance are given in Table II.

The color of the plants during growth was as follows: After 7 days' growth plants 55 to 72 were yellowish green, plants 91 to 108 and 73 to 90 were light green, plants 1 to 54 were dark green; after 12 days' growth plants 55 to 72 were light green and all others were of a good green color.

It is apparent from this experiment that the effect of the reaction of the solution depends somewhat on the quantity of iron supplied, this reaction being more evident in solutions containing a small amount of iron.

[1] This is not definitely proved by this experiment alone, but further work substantiated it.

TABLE II.—*Comparative weights of rice plants grown in acid and neutral solutions with three quantities of iron from ferrous sulphate*

Nutrient solution.	Quantity of iron per liter.	Flasks No.	Green weight of stalks and leaves.	Oven-dry weight of stalks and leaves.	Oven-dry weight of roots.	Average oven-dry weight of—		In dry substances of stalks and leaves, percentages of—		
						Stalks and leaves.	Whole plant.	Nitrogen (N)	Phosphoric acid (P_2O_5).	Iron (Fe_2O_3).
	Gm.		*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*P. c.*	*P. c.*	*P. c.*
Acid	0.002	1– 6	32. 05	4. 04	1. 04	....	....	....	....	.....
		7–12	32. 98	4. 12	1. 08	....	....	....	....	.....
		13– 18	31. 40	3. 93	. 92	4. 03	5. 04	4. 34	2. 31	0. 037
Do	.008, 0.004, 0.002	19– 24	30. 48	3. 90	. 96	....	....	....	....	.....
		25– 30	30. 00	3. 78	. 95	....	....	....	....	.....
		31– 36	34. 38	4. 18	. 91	3. 95	4. 89	4. 55	2. 20	. 037
Do	.008	37– 42	34. 72	4. 37	1. 32	....	....	....	....	.....
		43– 48	53. 10	6. 58	1. 92	....	....	....	....	.....
		49– 54	51. 18	6. 12	1. 80	5. 69	7. 37	4. 40	2. 02	. 034
Neutral	.002	55– 60	28. 48	3. 73	. 85	....	....	....	....	.....
		61– 66	26. 71	3. 34	. 79	....	....	....	....	.....
		67– 72	27. 72	3. 56	. 79	3. 54	4. 35	4. 56	2. 06	. 024
Do	.008, 0.004, 0.002	73– 78	36. 42	4. 40	1. 03	....	....	....	....	.....
		79– 84	37. 75	4. 69	1. 15	....	....	....	....	.....
		85– 90	28. 28	3. 62	. 84	4. 24	5. 24	4. 40	2. 04	. 020
Do	.008	91– 96	59. 18	7. 54	2. 17	....	....	....	....	.....
		97–102	.....	.....	....	....	....	....	....	.....
		103–108	59. 45	7. 49	2. 00	7. 52	9. 60	4. 28	1. 80	. 028

EXPERIMENT 3 (Sources of iron in acid, neutral, and alkaline solutions: Ferrous sulphate and ferric citrate).—This experiment was designed to observe the assimilation of iron in a solution containing calcium carbonate—that is, a slightly alkaline solution—and to compare the assimilability of ferric citrate with that of ferrous sulphate. The method used in the conduct of this experiment differed from that of other experiments only in the making of the nutrient solutions. In all other experiments reported the nutrient solutions were made up fresh 18 hours before the plants were inserted, and any residue remaining in the bottles from the previous lot was thrown away. In this experiment the nutrient solutions were made up fresh 18 hours beforehand, but whatever nutrient solution remained from the previous change was left in the bottles and added to the fresh solutions. This residue increased at times but never amounted to more than a quarter of the whole solution. It nevertheless affected the results.

Iron from both ferric citrate and ferrous sulphate was used at the rate of 0.002 gm. of iron per liter in all the cultures. The alkaline solutions with calcium carbonate contained 0.41 gm. of precipitated calcium carbonate per liter. One plant was grown in each flash. Six flasks were taken as a unit, and the units were triplicated for each treatment. The growths of the plants in the different solutions and a partial ash analysis of the stalks and leaves are given in Table III.

TABLE III.—*Comparative weights of rice plants grown in acid, neutral, and alkaline solutions with 0.002 gm. of iron per liter from ferrous sulphate or ferric citrate*

Nutrient solution.	Source of iron.	Flasks No.	Green weight of stalks and leaves.	Oven-dry weight of stalks and leaves.	Oven-dry weight of roots.	Average oven-dry weight of—		Composition of stalks and leaves.					
						Stalks and leaves.	Whole plant.	Carbon-free ash.	Silica (SiO_2).	Lime (CaO).	Magnesia (MgO).	Phosphoric acid (P_2O_5).	Iron (Fe_2O_3).
			Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Acid	Ferrous sulphate	1–6	38. 44	4. 57	1. 12	....	....	.....	....	....	....	....	.....
		7–12	37. 32	4. 49	1. 07	....	....	.....	....	....	....	....	.....
		13–18	32. 60	4. 05	1. 03	4. 37	5. 44	13. 90	0. 26	0. 74	1. 18	2. 03	0. 044
Do	Ferric citrate	19–24	46. 02	5. 13	1. 21	....	....	.....	....	....	....	....	.....
		25–30	40. 24	4. 63	1. 14	....	....	.....	....	....	....	....	.....
		31–36	43. 64	5. 13	1. 26	4. 96	6. 16	14. 41	. 13	. 75	1. 18	1. 97	. 039
Neutral	Ferrous sulphate	37–42	25. 25	2. 99	. 74	....	....	.....	....	....	....	....	.....
		43–48	29. 67	3. 48	. 83	....	....	.....	....	....	....	....	.....
		49–54	26. 66	3. 20	. 76	3. 22	3. 99	14. 19	. 28	. 79	1. 44	2. 03	. 028
Do	Ferric citrate	55–60	38. 14	4. 45	1. 06	....	....	.....	....	....	....	....	.....
		61–66	31. 78	3. 79	. 92	....	....	.....	....	....	....	....	.....
		67–72	38. 78	4. 45	1. 02	4. 23	5. 23	14. 23	. 22	. 78	1. 15	1. 94	. 024
Alkaline	Ferrous sulphate	73–78	13. 20	1. 76	. 70	....	....	.....	....	....	....	....	.....
		79–84	21. 05	2. 60	1. 07	....	....	.....	....	....	....	....	.....
		85–90	19. 15	2. 34	. 85	2. 23	3. 10	13. 64	. 39	. 96	1. 23	2. 17	. 020
Do	Ferric citrate	91–96	33. 30	3. 98	1. 25	....	....	.....	....	....	....	....	.....
		97–102	39. 72	4. 78	1. 51	....	....	.....	....	....	....	....	.....
		103–108	33. 64	4. 09	1. 43	4. 28	5. 68	14. 22	. 22	. 87	1. 16	2. 02	. 021

The color of the plants during growth was as follows: After 8 days growth plants 1 to 18 were a good green, and all others were strongly chlorotic; after 15 days growth plants 1 to 36 were a good green, 91 to 108 were slightly chlorotic, 55 to 72 were chlorotic, 73 to 90 and 37 to 54 were strongly chlorotic; after 23 days growth plants 1 to 36 were dark green, 91 to 108 and 55 to 72 were light green, 37 to 54 were a lighter green, and 73 to 90 were chlorotic.

In this experiment there was a greater difference in growth between the acid and neutral solutions with 0.002 gm. of iron per liter from ferrous sulphate than in the preceding experiment, probably due to the different method of preparing the solutions. The residual solutions not only contained less soluble iron than the freshly prepared solutions, but the precipitate already formed in the residual solutions probably affected the rate at which the iron in the freshly prepared solutions became insoluble.

RESULTS OF PRELIMINARY EXPERIMENTS

The three preliminary experiments showed that the effect of the reaction of the solution on growth and assimilation of iron depended somewhat on the quantity of iron added. With 0.002 gm. of iron per liter from ferrous sulphate, growth was greatest in the acid solution, but with 0.008 gm. of iron per liter, growth was greatest in the neutral solution.

Increasing the quantity of iron from 0.002 to 0.008 gm. per liter greatly increased the growth in both the acid and neutral solutions.

Analyses showed that plants grown in the different solutions did not differ appreciably in their percentages of nitrogen, phosphoric acid, lime, magnesia, or carbon-free ash, but did differ materially in the quantity of iron they contained. Plants grown in the acid solutions contained about 50 per cent more iron than plants grown in corresponding neutral or alkaline solutions.

In the course of this and other work it has been found that the color of the leaves is a pretty good indication of whether or not the plant is obtaining sufficient iron, although a lack of green is often attributable to other causes than a lack of iron. During growth, plants in the acid solution were the darkest green, in accordance with the percentages of iron found in the plants.

Used at the rate of 0.002 gm. of iron per liter ferric citrate was plainly a better source of iron than ferrous sulphate, especially in neutral and alkaline solutions.

That the growth in the different solutions was largely controlled by the supply of available iron seems proved by the color and analyses of the plants, and by the increased growth following an increase in quantity or change in the kind of iron added to the solutions.

FINAL EXPERIMENTS

EXPERIMENT 4 (Sources of iron in acid, neutral, and alkaline solutions: Ferrous sulphate and ferric citrate).—In this experiment 0.002 and 0.008 gm. of iron per liter from ferrous sulphate and 0.002 gm. of iron from ferric citrate were tested in the three different nutrient solutions. Two plants were grown in each flask and six flasks were taken as a unit, the units being duplicated for each treatment. The data of growth and the percentages of iron in the dry substance of the stalks and leaves are given in Table IV.

The color of the plants during growth was as follows: At 10 days plants 1 to 24 and 49 to 60 were a good green, all others were a yellowish green except plants 85 to 96, which were strongly chlorotic; at 20 days plants 1 to 24 and 49 to 72 were a good green, plants 25 to 48, 97 to 108, 73 to 84, and 97 to 108 were a slightly poorer color, and plants 85 to 96 were almost white.

The results of this experiment confirm those of the preliminary experiments in respect to (1) the superiority of ferric citrate to ferrous sulphate as a source of iron, (2) the relative growths in acid and neutral solutions with the two quantities of iron, and (3) the markedly higher percentages of iron in plants grown in the acid solutions. The new fact established was that increasing the iron from 0.002 to 0.008 gm. per liter in the alkaline solution depressed the growth of plants to a surprising degree. That the lack of growth in this solution was due to a lack of available iron is

proved by the pronounced chlorosis of the plants and by the results of experiment 10. Here, as well as in experiment 1, plants 85 to 96, which made practically no growth because of a lack of iron, contained a high percentage of iron in the dry substance. This point will be discussed later.

TABLE IV.—*Comparative weights of plants grown in acid, neutral, and alkaline solutions, with ferrous sulphate or ferric citrate as the source of iron*

Nutrient solution.	Source of iron.	Quantity of iron per liter.	Flasks No.	Green weight of stalks and leaves.	Oven-dry weight of stalks and leaves.	Oven-dry weight of roots.	Average oven-dry weight of—		Iron (Fe_2O_3) in dry stalks and leaves.
							Stalks and leaves.	Whole plant.	
		Gm.		*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Per ct.*
Acid	Ferrous sulphate	0.002	1–6	48.45	6.66	1.47	.....	.....	.....
Do	.....do	.002	7–12	45.85	6.42	1.46	6.54	8.01	.036
Do	.....do	.008	[a] 13–18	91.88	12.41	3.10	.....	.....	.....
Do	.....do	.008	19–24	83.29	10.94	2.65	11.68	14.56	.032
Do	Ferric citrate	.002	25–30	65.47	8.70	1.82	.....	.....	.....
Do	.....do	.002	31–36	60.07	8.47	1.83	8.59	10.42	.026
Neutral	Ferrous sulphate	.002	37–42	47.19	6.51	1.45	.....	.....	.....
Do	.....do	.002	43–48	42.39	5.92	1.35	6.21	7.61	.017
Do	.....do	.008	49–54	100.22	12.93	2.97	.....	.....	.....
Do	.....do	.008	55–60	100.31	13.05	2.90	12.99	15.93	.023
Do	Ferric citrate	.002	61–66	62.07	8.32	1.83	.....	.....	.....
Do	.....do	.002	67–72	58.60	7.85	1.79	8.09	9.90	.015
Alkaline	Ferrous sulphate	.002	73–78	45.18	6.17	1.63	.....	.....	.....
Do	.....do	.002	79–84	42.13	6.22	1.68	6.20	7.86	.017
Do	.....do	.008	85–90	.93	.21	.11	.....	.....	.....
Do	.....do	.008	91–96	1.07	.23	.11	.22	.33	.049
Do	Ferric citrate	.002	97–102	63.28	8.33	2.20	.....	.....	.....
Do	.....do	.002	103–108	61.85	8.29	2.11	8.31	10.47	.012

[a] Results calculated from five flasks.

EXPERIMENT 5 (Ferrous sulphate in neutral solution with and without carbon black).—Data of growth in the different solutions seemed to point overwhelmingly to the iron supply as the factor controlling growth; nevertheless, it was possible that in some cases the precipitate in the solutions affected growth aside from any influence of the precipitate on the amount of soluble iron. After the addition of iron salts to the nutrient solutions a precipitate was formed which varied according to the source of the iron and the nutrient solution, but was greater as the amount of iron added became larger. In the preceding experiments a marked increase in growth was produced by increasing the iron from 0.002 to 0.008 gm. per liter in the neutral solution. It was possible that the greater precipitate in the solution with 0.008 gm. of iron favored growth in removing by absorption traces of heavy metals or other substances present in the distilled water. As carbon black and ferric hydroxid have been found to improve the quality of distilled water in this way, the following test was conducted.

To two lots of solutions carbon black was added at the rate of 0.0432 gm. per liter. This is about double the weight of the precipitate in the ordinary neutral solution with 0.008 gm. of iron per liter from ferrous sulphate. Two other solutions were made up using distilled water that had previously been treated with 0.086 gm. of carbon black per liter and then filtered. The neutral nutrient solution was used in all sets, and ferrous sulphate was the source of the iron. Two plants were grown in each flask, six flasks were taken as a unit, and the units duplicated for each treatment. The results of the test are given in Table V.

TABLE V.—*Comparative weights of rice plants grown in neutral nutrient solution with iron from ferrous sulphate and also with distilled water treated with carbon black*

Kind of distilled water used in nutrient solution.	Quantity of iron per liter.	Flasks No.	Green weight of stalks and leaves.	Oven-dry weight of stalks and leaves.	Oven-dry weight of roots.	Average oven-dry weight of—	
						Stalks and leaves.	Whole plant.
	Gm.		*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Ordinary distilled water	+0.002	1–6	62.19	8.27	1.86	.....	.....
		7–12	65.07	8.48	1.92	8.38	10.27
Do	+ .008	13–18	128.62	16.31	3.73	.....	.....
		19–24	137.50	16.73	4.01	16.52	20.39
Ordinary distilled water + carbon black.	+ .002	25–30	58.56	7.65	1.69	.....	.....
		31–36	57.01	7.55	1.71	7.60	9.30
Do	+ .008	37–42	139.40	17.54	3.54	.....	.....
		43–48	127.70	15.91	3.47	16.73	20.24
Do	+ .002	49–54	88.20	11.10	2.56	.....	.....
		55–60	88.10	10.97	2.49	11.04	13.57
Do	+ .008	61–66	148.53	18.49	4.42	.....	.....
		67–72	147.87	18.62	4.65	18.56	23.10

During growth lots with 0.002 gm. of iron per liter were of a slightly poorer color than lots with 0.008 gm., but no differences in color were apparent between the lots with different distilled waters or with carbon black.

It is evident that the addition of carbon black to the nutrient solutions with 0.002 gm. of iron slightly depressed rather than increased the yield while carbon in the solution with 0.008 gm. of iron had no effect on the yield. Treating the distilled water with carbon and then filtering increased the yield in the solution with 0.002 gm. of iron by 32 per cent and in the solution with 0.008 gm. by 12 per cent.

Previously treating the distilled water with carbon increased the growth less in the solution with 0.008 gm. of iron than in the solution with 0.002 gm., possibly because the formation of the larger flocculent precipitate of iron in the former solution acted similarly to carbon in removing injurious substances from the distilled water. Probably the adsorption of the other salts prevented the carbon when added directly to the nutrient

solution from adsorbing to any extent these injurious substances. The slight depression in growth produced by carbon in the solution with 0.002 gm. of iron may well be due to slightly diminishing the amount of soluble iron in the solution by adsorption.

It thus seems possible that the larger flocculent iron precipitate which existed in the solution with 0.008 gm. of iron per liter may have increased growth to some extent by improving the distilled water; but only a small part of the greater growth produced by increasing the iron from 0.002 to 0.008 gm. can be attributed to that cause.

EXPERIMENT 6 (Source of iron in acid, neutral, and alkaline solutions: Ferric chlorid).—The results with ferrous sulphate as the source of iron may well be complicated by the fact that both ferrous and ferric iron doubtless existed in the solution unless the ferrous iron was oxidized by the plants' roots (13). In the very dilute solutions it was, of course, impossible to tell how much of each kind of iron was present. In the following experiment with ferric chlorid as the source of iron naturally ferric iron only was present.

Ferric chlorid was added to the acid, neutral, and alkaline solutions so as to furnish 0.002 and 0.008 gm. of iron per liter. Two seedlings were grown in each flask, six flasks were taken as a unit, and the units triplicated for each treatment. The growth of plants and percentages of iron in the dry substance are given in Table VI.

TABLE VI.—*Comparative weights of rice plants grown in acid, neutral, and alkaline solutions, with ferric chlorid as the source of iron*

Nutrient solution.	Quantity of iron per liter.	Flasks No.	Green weight of stalks and leaves.	Oven-dry weight of stalks and leaves.	Oven-dry weight of roots.	Average oven-dry weight of—		Iron (Fe_2O_3) in dry stalks and leaves.
						Stalks and leaves.	Whole plant.	
	Gm.		*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Per ct.*
Acid	0.002	1–6	26.50	3.89	0.75	.....	.....	.....
		7–12	32.37	4.59	.87	.....	.....	.....
		13–18	24.96	3.46	.68	3.98	4.75	0.025
Do	.008	19–24	37.67	5.32	.97	.....	.....	.....
		25–30	39.43	5.49	1.10	.....	.....	.....
		31–36	34.62	4.78	.94	5.20	6.20	.026
Neutral	.002	37–42	29.72	4.16	.83	.....	.....	.....
		43–48	28.22	3.81	.80	.....	.....	.....
		49–54	29.56	3.83	.85	3.93	4.76	.022
Do	.008	55–60	38.12	5.17	1.03	.....	.....	.....
		61–66	41.76	5.82	1.21	.....	.....	.....
		67–72	40.70	5.65	1.16	5.55	6.68	.026
Alkaline	.002	73–78	6.96	1.09	.32	.....	.....	.....
		79–84	6.97	1.10	.33	.....	.....	.....
		85–90	6.33	.97	.31	1.05	1.37	.022
Do	.008	91–96	10.03	1.51	.42	.....	.....	.....
		97–102	9.26	1.36	.37	.....	.....	.....
		103–108	7.81	1.22	.33	1.36	1.73	.023

The color of the plants during growth was as follows: At 10 days plants 19 to 36 and 55 to 72 were a good green, 1 to 18 were a lighter green, 37 to 54 were a yellowish green, 73 to 108 had a still poorer color; at 20 days plants 19 to 36 and 55 to 72 were a fair green, 1 to 18 and 37 to 54 were markedly chlorotic, 73 to 108 were more chlorotic; at 30 days plants 19 to 36 and 55 to 72 were of slightly poor color, in 1 to 18 and 37 to 54 color was improved though still chlorotic, 73 to 108 were strongly chlorotic. The color and growth of plants were, on the whole, noticeably inferior to that of plants grown with ferrous sulphate as the source of iron.

The growths made in the acid and neutral solutions were approximately equal and much superior to those in the alkaline solution. Growth was markedly increased in all three solutions by increasing the iron from 0.002 to 0.008 gm. per liter.

The results differ from those of previous experiments with ferrous sulphate because in acid and neutral solutions growth was increased less by increasing the iron from 0.002 to 0.008 gm.; with 0.002 gm. of iron growth was much less in the alkaline solution relative to growth in neutral and acid solutions; increasing the iron from 0.002 to 0.008 gm. in the alkaline solution measurably increased growth instead of markedly depressing it.

The percentages of iron in the dry substance of stalks and leaves varied very little between plants from the different solutions. In each case plants grown in solutions with 0.008 gm. of iron per liter contained very slightly more iron than those grown in similar solutions with 0.002 gm. of iron. It will be noted that there was not such a marked increase in the assimilation of iron from the acid solution as in tests with ferrous sulphate.

EXPERIMENT 7 (Source of iron in acid, neutral, and alkaline solutions: Ferric citrate).—Ferric citrate as a source of iron was compared with ferrous sulphate in experiments 3 and 4, but only 0.002 gm. of iron per liter was used. In this test both 0.002 and 0.008 gm. of iron per liter were compared in the different solutions.

Three seedlings were grown in each flask, but 200 c. c. flasks were used only during the first 15 days, 500 c. c. flasks during the next 15 days, and 1,000 c. c. flasks during the last 10 days of growth. Six flasks were taken as a unit and the units duplicated for each treatment. The growth of plants and percentages of iron in dry stalks and leaves are shown in Table VII.

During the first 30 days all plants were of a good green color, except No. 61 to 72, which were yellowish green. All plants had a good color during the last 10 days.

As in the previous tests with ferric citrate, the growth was equal in acid, neutral, and alkaline solutions with 0.002 gm. of iron per liter. With 0.008 gm. of iron per liter growth was practically equal in the acid and neutral solutions, but much less in the alkaline. Growth was

markedly increased in acid and neutral solutions, but was unaffected in the alkaline solution by increasing the iron from 0.002 to 0.008 gm. per liter.

TABLE VII.—*Comparative weights of rice plants grown in acid, neutral, and alkaline solutions, with ferric citrate as the source of iron*

Nutrient solution.	Quantity of iron per liter.	Flasks No.	Green weight of stalks and leaves.	Oven-dry weight of stalks and leaves.	Oven-dry weight of roots.	Average oven-dry weight of—		Iron (Fe_2O_3) in dry stalks and leaves.
						Stalks and leaves.	Whole plant.	
	Gm.		*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Per ct.*
Acid	0.002	1–6	87.90	11.02	2.13	.....	.....	.....
		7–12	87.01	10.96	2.15	10.99	13.13	0.019
Do	.008	13–18	124.45	17.42	3.28	.....	.....	.....
		19–24	143.40	18.08	3.65	17.75	21.27	.025
Neutral	.002	25–30	87.31	11.19	2.23	.....	.....	.....
		31–36	84.43	11.08	2.28	11.14	13.40	.016
Do	.008	37–42	130.95	16.35	3.27	.....	.....	.....
		43–48	110.12	13.73	2.60	15.04	17.98	.020
Alkaline	.002	49–54	91.16	11.55	2.34	.....	.....	.....
		55–60	88.65	11.28	2.55	11.42	13.87	.016
Do	.008	61–66	70.30	8.82	2.11	.....	.....	.....
		67–72	91.64	11.75	2.82	10.29	12.76	.020

The percentages of iron in plants grown in acid solutions were slightly greater than in plants grown in neutral and alkaline solutions. The iron contents of plants from all three solutions were slightly increased by increasing the iron from 0.002 to 0.008 gm. per liter.

EXPERIMENT 8 (Source of iron, in acid, neutral, and alkaline solutions: Ferric tartrate).—This experiment was conducted to see whether other organic iron compounds would show the same availability as ferric citrate. The two quantities of iron from ferric tartrate were used in the three nutrient solutions. Two plants were grown in each flask, six flasks were taken as a unit, and the units triplicated for each treatment. The growth of plants and percentages of iron in the stalks and leaves are given in Table VIII.

During the growth the plants were all of good color except No. 37 to 54 and 73 to 90, which were slightly yellowish from the fifteenth to thirtieth day, but of good color the rest of the time.

With 0.002 gm. of iron per liter growth was considerably better in the acid solution than in the neutral or alkaline. With 0.008 gm. of iron per liter growth was practically equal in all three solutions, though possibly there was a slight depression in the neutral solution. Increasing the iron did not increase growth in the acid solution, but did increase it in the neutral and alkaline solutions to a small extent.

The percentages of iron in plants grown in the acid solutions were very slightly greater than in plants grown in the neutral and alkaline solutions.

The iron contents of all plants except those grown in the alkaline solution were increased by increasing the iron from 0.002 to 0.008 gm. of iron per liter.

TABLE VIII.—*Comparative weight of rice plants grown in acid, neutral, and alkaline solutions, with ferric tartrate as the source of iron*

Nutrient solution.	Quantity of iron per liter.	Flasks No.	Green weight of stalks and leaves.	Oven-dry weight of stalks and leaves.	Oven-dry weight of roots.	Average oven-dry weight of—		(Fe_2O_3) in dry stalks and leaves.
						Stalks and leaves.	Whole plant.	
	Gm.		*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Per ct.*
Acid	0.002	1– 6	67.81	8.97	2.20	.....	.....	.....
		7– 12	56.61	7.92	1.93	.....	.....	.....
		13– 18	62.48	8.65	2.04	8.51	10.57	0.022
Do	.008	19– 24	52.05	6.89	1.90	.....	.....	.....
		25– 30	67.27	8.91	2.19	.....	.....	.....
		31– 36	68.46	9.14	2.26	8.31	10.43	.030
Neutral	.002	37– 42	53.58	7.01	1.52	.....	.....	.....
		43– 48	47.95	6.41	1.47	.....	.....	.....
		49– 54	51.80	6.91	1.58	6.78	8.30	.020
Do	.008	55– 60	57.36	7.58	1.81	.....	.....	.....
		61– 66	56.86	7.72	1.78	.....	.....	.....
		67– 72	63.70	8.60	2.01	7.97	9.84	.025
Alkaline	.002	73– 78	50.22	6.75	1.84	.....	.....	.....
		79– 84	48.17	6.52	1.92	.....	.....	.....
		85– 90	43.01	6.06	1.70	6.44	8.26	.019
Do	.008	91– 96	58.95	8.04	2.40	.....	.....	.....
		97–102	63.13	8.56	2.32	.....	.....	.....
		103–108	62.90	8.46	2.17	8.35	10.65	.022

EXPERIMENT 9 (Source of iron in acid and neutral solutions: Dialyzed iron).—Dialyzed iron was tested as a source of iron in acid and neutral solutions. From the previous experiments it seemed evident that this form of iron would be of very low availability, but as it has been recommended as a source of iron in certain nutrient solutions it was thought advisable to make the test. The comparison between dialyzed iron and ferrous sulphate in the neutral solution was carried out at one time and the comparison between the neutral and acid solutions with dialyzed iron at another time. The two tests are combined in one table for conciseness.

Two plants were grown in each flask, six flasks were taken as a unit, and the unit duplicated for each treatment. Plants in flasks 49 to 84, the comparison between acid and neutral solutions, were grown but 25 days; all others were grown 40 days. The data of growth are given in Table IX.

All plants except No. 25 to 48 were markedly chlorotic at all times.

Dialyzed iron apparently afforded practically no available iron when used in the neutral solution at the rate of either 0.002 or 0.008 gm. of iron per liter. In the acid solution it was slightly more available than in the neutral, although utterly inadequate for the needs of the plant.

TABLE IX.—*Comparative weights of rice plants grown in acid and neutral solutions, with dialyzed iron as the source of iron*

Nutrient solution.	Quantity of iron. per liter.	Source of iron.	Flasks No.	Green weight of stalks and leaves.	Oven-dry weight of stalks and leaves.	Oven-dry weight of roots.	Average oven-dry weight of—	
							Stalks. and leaves.	Whole plant.
				Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Neutral	0.002	Dialyzed iron	1–6	0.67	0.15	0.054	.....	.....
			7–12	.61	.16	.054	0.16	0.21
Do	.008	do	13–18	.63	.17	.054	.....	.....
			19–24	.61	.16	.054	.16	.22
Do	.002	Ferrous sulphate.	25–30	62.19	8.27	1.86	.....	.....
			31–36	65.07	8.48	1.92	8.38	10.27
Do	.008	do	37–42	128.62	16.31	3.73	.....	.....
			43–48	137.50	16.73	4.01	16.52	20.39
Do	.008	Dialyzed iron	49–54	.90	.16	.062	.....	.....
			55–60	.99	.19	.062	.18	.24
Acid	.008	do	73–78	4.38	.67	.182	.....	.....
			79–84	4.26	.64	.176	.66	.84

EXPERIMENT 10 (Effect of applying ferrous sulphate to leaves of plants grown in solutions where iron was markedly unavailable).—In experiment 4 rice failed to grow appreciably in the alkaline solution when 0.008 gm. of iron per liter from ferrous sulphate was used, although it made a fair growth with 0.002 gm. of iron, and in the previous experiment no perceptible growth was made in the neutral solution with dialyzed iron. In order to be certain that the inability of the plants to grow in these solutions was due to a lack of available iron, the following test was made of applying iron to the leaves.

The leaves of plants in these two solutions were brushed with ferrous sulphate, and two control lots were not brushed. The specially treated plants were brushed once with a 0.1 per cent solution of ferrous sulphate, twice with a 0.2 per cent solution, and three times with a 0.4 per cent solution. This was done so that no iron could get from the leaves to the solution. Two plants were grown in each flask, six flasks were taken as a unit, and the units triplicated for each treatment. The plants were grown but 25 days, as results were then decisive, the unbrushed plants having ceased to grow perceptibly some time before. The data on growth are given in Table X.

The leaves brushed with the ferrous-sulphate solution became green in two or three days. New leaves appearing at intervals between the brushings were strongly chlorotic, but became green quickly when treated with the solution. Plants 1 to 12 and 25 to 36, ones that were not brushed at any time, were always strongly chlorotic and made no appreciable growth. The comparative growths of the brushed and unbrushed plants show decisively that the inability of plants to grow in these solutions was due to a lack of available iron.

TABLE X.—*Comparative weights of rice plants grown in two solutions where the iron was unavailable, but with the leaves treated with ferrous sulphate*

Nutrient solution.	Quantity of iron per liter.	Source of iron.	Treatment.	Flasks No.	Green weight of stalks and leaves.	Oven-dry weight of stalks and leaves.	Oven-dry weight of roots.	Average oven-dry weight of—	
								Stalks and leaves.	Whole plant.
	Gm.				*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Alkaline..	0.008	Ferrous sulphate.	Leaves not brushed..	1-6	2.47	0.39	0.131	....	....
				7-12	2.48	.39	.12	0.39	0.52
Do.....	.008	..do.....	Leaves brushed with ferrous sulphate.	13-18	14.63	2.14	.630	....	....
				19-24	15.95	2.32	.602	2.23	2.85
Neutral ...	.008	Dialyzed iron.	Leaves not brushed..	25-30	.90	.16	.062	....	....
				31-36	.99	.19	.062	.18	.24
Do.....	.008	...do.....	Leaves brushed with ferrous sulphate.	37-42	11.44	1.69	.463	....	....
				43-48	9.40	1.40	.383	1.50	1.92

EXPERIMENT 11 (Effect of increasing the phosphates in the neutral solution).—Since there is always more or less iron in the nutrient solutions precipitated as phosphates, it was important to see whether increasing the phosphates in the solution would affect the growth of plants. In this test the neutral solution used in the preceding experiments was compared with a solution which contained double the quantity of mono- and dibasic potassium phosphate, but which was otherwise similar. Two tests were run at different times, one using ferric chlorid as the source of iron and one using ferric citrate. For the sake of conciseness they are combined in one table.

In the test with ferric chlorid two plants were grown in each flask, 6 flasks were taken as a unit and the units triplicated for each treatment. In the test with ferric citrate one plant was grown in each flask, 12 flasks were taken as a unit, and the units duplicated for each treatment. The data on the growth of these plants are given in Table XI.

The ferric-chlorid plants were all of rather poor color, those with 0.002 gm. of iron per liter being poorer than those with 0.008 gm., but no differences were apparent between plants in the ordinary and double-phosphate solutions. The ferric-citrate plants in both ordinary and double-phosphate solutions were of good color at all times.

In regard to growth and iron content, there were no appreciable differences between plants in the ordinary and double-phosphate solutions, although the very slight differences in growth were always in favor of the ordinary solution.

TABLE XI.—*Comparative weights of rice plants grown in neutral solution with phosphates doubled, with ferric chlorid or citrate source of iron*

Nutrient solution.	Quantity of iron per liter.	Source of iron.	Flasks no.	Green weight of stalks and leaves.	Oven-dry weight of stalks and leaves.	Oven-dry weight of roots.	Average oven-dry weight of—		Iron (Fe_2O_3) in dry stalks and leaves.
							Stalks and leaves.	Whole plant.	
				Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Per ct.*
Neutral	0.002	Ferric chlorid	1–6	28.22	3.54	0.68			
			7–12	34.30	4.24	.84			
			13–18	28.76	3.63	.69	3.80	4.54	0.020
Neutral, with double phosphates.	.002	do	19–24	29.84	3.78	.74			
			25–30	27.43	3.49	.68			
			31–36	29.42	3.89	.78	3.72	4.45	.021
Neutral	.008	do	37–42	42.12	5.21	1.20			
			43–48	37.24	4.56	1.03			
			49–54	38.16	4.76	1.10	4.84	5.95	.023
Neutral, with double phosphates.	.008	do	55–60	39.23	4.88	.99			
			61–66	37.30	4.72	.93			
			67–72	34.51	4.39	.90	4.66	5.60	.022
Neutral	.008	Ferric citrate	1–12	202.36	29.95	7.41			
			13–24	192.20	28.72	7.11	29.34	36.65	.019
Neutral, with double phosphates.	.008	do	25–36	181.78	27.33	6.74			
			37–48	189.34	28.85	7.21	28.09	35.07	.021

SOLUBILITY OF IRON IN THE NUTRIENT SOLUTIONS

An attempt was made to estimate the soluble iron in some of the nutrient solutions by filtering the solutions to remove precipitated iron and determining iron in the filtrate. Solutions 1 to 9 (used in experiment 4) were analyzed after they had stood various lengths of time both with and without plants growing in them. Iron in all the solutions was determined by the colorimetric method with potassium sulphocyanate, but solutions 1, 2, 4, 5, 7, and 8 without the growth of plants were also analyzed by the method of Stokes and Cain. Practically the same results were obtained by the two methods. The results are shown in Table XII. Figures for iron in the solutions without plants are the averages of two or more determinations, while the figures for iron in solutions in which plants had grown represent single determinations. The results for solution 5 without plants are not trustworthy, as this solution could not be filtered absolutely clear by any device the writers considered permissible to employ.

In a comparison of the growth of plants in the different solutions with the amount of iron in the filtered solutions, almost as many discrepancies as agreements are apparent. One striking discrepancy is the small amount of iron in the filtrate of No. 3 and the good growth of plants in this solution. Repeated analyses of the precipitate left on the filter

showed that practically all the iron added to this solution was precipitated. As this solution evidently had less precipitate than some others, the character of the precipitate probably varied somewhat in the different solutions. The amount of iron present in the filtered solutions in which plants had grown also failed to correspond to the growth of plants.

TABLE XII.—*Quantity of iron (in grams) in filtered solutions at different times after addition of iron to solution, with and without growth of plants in the solution*

Solution No.	Nutrient solution.	Source of iron.	Iron per 1,000 liters.	Quantity of iron in 1,000 liters of filtered solution.							
				No plants in solutions.			Plants grown in solutions.				
				24 hours after iron had been added.	72 hours after iron had been added.	120 hours after iron had been added.	After 10-day-old plants had been grown in solution for 96 hours.	After 14-day-old plants had been grown in solution for 96 hours.	After 30-day-old plants had been grown in solution for 48 hours.	After 35-day-old plants had been grown in solution for 48 hours.	After 37-day-old plants had been grown in solution for 96 hours.
1	Acid....	Ferrous sulphate...	2.0	1.35	1.30	0.90	0.12	0.07	0.05	0.05	0.03
2	.....do..	.....do.....	8.0	5.00	4.20	3.70	.30	.30	.43	.23	.07
3	.....do..	Ferric citrate.....	2.0	< .40	.0	.0	.12	< .03	.07	.05	.03
4	Neutral.	Ferrous sulphate...	2.0	1.35	1.30	1.00	.67	.49	1.27	1.13	.80
5	.....do..	.....do.....	8.0	1.00	.70	.50	.07	.03	.17	.10	.07
6	.....do..	Ferric citrate.....	2.0	1.65	1.20	1.00	.80	.87	1.63	1.33	.80
7	Alkaline	Ferrous sulphate...	2.0	1.15	.80	.40	.37	.40	1.33	.86	.60
8	.....do..	.....do.....	8.0	.....	....	.03	< .07	.05	.18	....	....
9	.....do..	Ferric citrate.....	2.0	1.40	.90	.40	.57	.57	1.00	.93	.53

The growth of plants did not agree with the quantity of iron found in the filtered solutions because, without doubt, colloidal iron, as well as truly soluble iron, was present. Previous work having shown that colloidal iron was not available for rice (6), the determinations in Table XII did not represent the available iron. At this great dilution it was, of course, impossible to distinguish analytically between colloidal and soluble iron or between ferrous and ferric iron. While the existence of colloidal iron could not be definitely demonstrated in these solutions, it is well known that most ferric salts in dilute solutions are more or less completely hydrolytically dissociated into colloidal ferric hydroxid and the acid. Moreover, a test showed that dialyzed iron could not be distinguished from distilled water with regard to color or filtration when used in somewhat greater concentration than iron was present in the above filtrates.

In solutions where ferric iron was used iron was probably present in the following forms: (1) As precipitated ferric phosphate and hydroxid, (2)

as colloidal ferric hydroxid, (3) as soluble undissociated iron compounds, and (4) as ionized iron. There was probably a balance between these forms of iron, as after filtering the nutrient solutions more precipitate formed on standing a short time.[1] From determinations of iron in the filtered nutrient solutions it is evident that more or less half the iron was precipitated as phosphate and hydroxid. The amount and composition of this precipitate probably varied somewhat in the different solutions (9). The greater part of the remaining iron was probably present as colloidal ferric hydroxid. The available iron, which included the soluble undissociated and ionized iron, was undoubtedly extremely small and was governed chiefly by the completeness of the hydrolysis of the dissolved iron. The amount of iron hydrolized would depend on the reaction of the solution, being less in acid solutions, and would also depend on the form in which iron was added, being less with the less ionized organic salts. The effect of the form of iron and the reaction of the solution on the assimilation of iron by rice is thus easily comprehensible.

While the amount of soluble or available iron could not be determined analytically, some idea of the very small amount present could be obtained by comparing the amount of iron absorbed by the plants with the total amount of iron added in the volume of solution available during growth. This calculation showed that the plants absorbed only one-fifteenth to one two-hundredth part of the iron supplied, even in solutions where growth was obviously inhibited by lack of iron. If it is assumed that one fifteenth to one two-hundredth part of the iron added to the solutions was in a soluble condition, the concentration of the soluble iron in some cases would have been from 0.13 to 0.01 part per million. A more probable assumption is that the concentration of soluble iron present at any one time was even lower and that as iron was removed by the plant more went into solution. It thus appears that in some cases the amount of iron in true solution must have been too small for slight differences to be accurately determined. It is evident that in certain nutrient solutions rice can assimilate sufficient iron when the concentration of soluble iron is probably less than 1 part in 10,000,000.

Comparative analyses in Table XII of solutions in which plants had and had not grown show that at least the amount of colloidal iron was notably diminished by the growth of plants in the solution. The colloidal iron was evidently precipitated.

SUMMARY OF EXPERIMENTAL RESULTS

The results of the previous culture experiments showed plainly that rice in nutrient solutions was not particularly sensitive to an acid or alkaline reaction *per se*. Apparently the reaction of the nutrient solution affected the growth of rice only through influencing the availability of the

[1] The precipitates in these dilute nutrient solutions were iron compounds, as before the addition of iron salts the solutions were perfectly clear.

iron, since the relative growths made in acid, neutral, and alkaline solutions depended on the kind and quantity of iron supplied. This is well shown by Table XIII, which summarizes the relative growths of stalks and leaves made in the three solutions with different kinds and quantities of iron. The growth of stalks and leaves [1] made in the acid solution is always taken as 100; growths in the neutral and alkaline solutions are expressed relative to 100.

TABLE XIII.—*Relative growths of rice plants in acid, neutral, and alkaline solutions with different sources and amounts of iron*

Source of iron in nutrient solutions.	Iron per liter added to nutrient solutions.	Relative growths in—			Table from which data were calculated.
		Acid solution.	Neutral solution.	Alkaline solution.	
	Gm.				
Ferrous sulphate	0.002	100	88	.....	II
Do	.....	100	74	51	III
Do	.....	100	95	95	IV
Do	.008	100	105	.....	I
Do	.004				
Do	.002	100	107	.....	II
Do	.008	100	132	.....	II
Do	.....	100	111	2	IV
Ferric chlorid	.002	100	99	26	VI
Do	.008	100	107	26	VI
Ferric citrate	.002	100	85	86	III
Do	.....	100	94	97	IV
Do	.....	100	101	104	VII
Do	.008	100	85	58	VII
Ferric tartrate	.002	100	80	76	VIII
Do	.008	100	96	100	VIII
Dialyzed iron	.008	100	27	.....	IX

It is evident that with 0.002 gm. of iron per liter, growth was more or less best in the acid solution, while with 0.008 gm. of iron per liter growth was best in the neutral solution with some forms of iron. Growth with most, but not all, forms and quantities of iron was strikingly inferior in the alkaline solution.

The effect on growth of increasing the quantity of iron in the different solutions is significant in throwing light on the availability of the iron in these solutions. In Table XIV are shown the extents to which growth was increased or decreased by increasing the iron in the three solutions from 0.002 to 0.008 gm. per liter. The growth made in each solution (acid, neutral, or alkaline) with 0.002 gm. of iron per liter is taken as 100, and the growth made with 0.008 gm. of iron per liter in these solutions is expressed relative to 100.

[1] In this and previous comparisons more significance was attached to the weight of stalks and leaves than to the weight of the whole plant. The weights given for the roots must have been very slightly in excess of the true values, as the roots were always more or less contaminated with a precipitate that could not be removed by washing. Also, the root growth relative to top growth is markedly influenced in some cases by an insufficiency of a mineral nutrient.

TABLE XIV.—*Relative growths of rice plants with 0.002 and 0.008 gm. of iron per liter in the different solutions*

Source of iron in nutrient solution.	Growth [a] with 0.008 gm. of iron per liter in—			Table from which data were calculated.
	Acid solution.	Neutral solution.	Alkaline solution.	
Ferrous sulphate	141	212	.....	II
Do	179	209	4	IV
Do	.....	197	.....	V
Ferric chlorid	131	141	140	VI
Do	.....	127	.....	XI
Dialyzed iron	.....	100	.....	IX
Ferric citrate	162	135	90	VII
Ferric tartrate	98	118	130	VIII

[a] Growth with 0.002 gm. of iron per liter=100.

In most cases an increase in the amount of iron added to the solution produced a marked increase in growth. With ferric tartrate in the acid solution an increase in iron produced no increase in growth, as apparently the smaller quantity of iron was adequate. In the alkaline solution with ferric citrate and with ferrous sulphate there were, respectively, no increase and a striking depression in growth, following an increase in the amount of iron added to the solution. Increasing the ferric citrate in this solution apparently did not increase the soluble iron and increasing the ferrous sulphate must have decreased it, as the smaller amounts of iron were also inadequate in these solutions.

From the growth of the plants it appears that ferrous sulphate, ferric citrate, or ferric tartrate used in proper quantities afforded sufficient iron in acid or neutral solutions. With the two quantities of iron used ferric chlorid was inferior as a source of iron, and dialyzed iron was utterly inadequate. Ferric tartrate was the only form of iron tried which appeared to afford sufficient iron in the solution alkaline with calcium carbonate.

The addition of carbon black to the neutral nutrient solution with 0.002 gm. of iron per liter very slightly depressed growth, but carbon black in the solution with 0.008 gm. of iron did not affect the yield. Treating the distilled water with carbon and filtering previous to its use in the nutrient solution increased growth considerably over that in the ordinary nutrient solution.

Doubling the phosphates in the neutral nutrient solution did not measurably affect growth when either ferric chlorid or citrate was used as the source of iron.

In all the cultural tests, plants grown in acid solutions contained more iron than those grown in corresponding neutral or alkaline solutions. Plants grown in neutral solutions contained more iron than those grown

in corresponding alkaline solutions when ferrous sulphate or ferric chlorid was the source of iron, but about equal percentages when ferric citrate or tartrate was the source of iron. With ferric chlorid, citrate, or tartrate an increase in the quantity of iron from 0.002 to 0.008 gm. of iron per liter in acid, neutral, or alkaline solution raised the percentage of iron in the plant. Increasing the ferrous sulphate in the acid solution did not increase the percentage of iron in the plants, while in the neutral solution it did have this effect. The percentages of nitrogen, phosphoric acid, lime, magnesia, and carbon-free ash in plants grown in six different solutions did not vary appreciably. The relative percentages of iron in the plants thus agreed with relative growths in showing that the amount of available iron in most solutions was the main factor controlling growth.

In regard to the percentages of iron in the plants two anomalous features are apparent in the preceding tables: (1) The percentages of iron in plants supplied with ferrous sulphate were higher than in plants supplied with ferric citrate, and (2) in two cases plants which made no growth because of a lack of iron contained as high a percentage of iron as plants of good growth.

Experiments 3 and 4 each afford a comparison of ferric citrate and ferrous sulphate in the three different nutrient solutions. In all cases 0.002 gm. of iron per liter from ferric citrate produced a better growth of plants with a lower percentage of iron than did the same quantity of iron from ferrous sulphate. As tests 2, 3, 4, and 7 show, neither of these forms of iron, supplied at this rate, furnished sufficient iron for the maximum needs of the plant. Therefore the higher percentages of iron in the ferrous-sulphate plants could not have been due to excessive consumption. About the only explanation of the anomaly that occurs to the writers is that the ferrous-sulphate plants contained a certain amount of iron which was ineffective in the metabolism of the plant. In the solutions to which ferrous sulphate was added undoubtedly both ferrous and ferric iron existed in solution, while in the solutions with ferric citrate there was only ferric iron. It is possible that in the solutions with ferrous sulphate the plants absorbed both ferrous and ferric iron and that the ferrous iron was not so effective in the plant as the ferric iron. The ferrous-sulphate plants might therefore contain a certain amount of effective and non-effective iron. This explanation is somewhat doubtful, as it is not, to the knowledge of the writers, supported by similar well-established facts.

The second irregularity is the high percentage of iron in plants 1 to 12, experiment 1, and plants 85 to 96, experiment 4, the former being grown without the addition of any iron to the solution, and the latter obtaining practically no iron from the alkaline solution, as proved by experiment 10. It is, of course, possible that the high percentages of iron were due to contamination, but this is thought not to be the cause. The plants made practically no growth and were so strongly chlorotic that they

could have elaborated scarcely any organic matter; the leaves were especially thin and often withered as soon as formed. It is possible that the high percentage of iron in the dry substance might have been due to the fact that enough iron was not present in the plant to start the production of carbohydrates, which would have lowered the percentage. While this explanation opens up several points not covered by the present investigation, it is supported somewhat by the following test (Table XV, first test), where plants grown for 13 days with iron and then for 13 days without iron contained a lower percentage of iron than plants grown the full 26 days without iron.

TABLE XV.—*Percentages of iron in rice plants grown with and without iron*

Test and treatment of plants.	Average dry weight of stalks and leaves per plant.	Percentage of iron (F_2O_3), in dry stalks and leaves.
First test:		
Grown for 13 days, without the addition of iron to the solution	*Gm.* 0.017	0.020
Grown for 26 days, without the addition of iron to the solution	.018	.026
Grown for 13 days, without the addition of iron to the solution and then for 13 days with iron	.097	.020
Grown for 26 days, with the addition of iron to the solution	.311	.027
Second test:		
Grown 13 days, without the addition of iron to the solution	.016	.015
Grown 26 days, without the addition of iron to the solution	.019	.024
Grown 40 days, without the addition of iron to the solution	.026	.049
Grown 40 days, with the addition of iron to the solution	1.547	.030

In the first test plants grown 26 days without iron contained a higher percentage of iron than those grown 13 days, although there was practically no increase in growth. This peculiarity was repeated in a second test (Table XV).

From the regularity of these results it appears they were due to peculiarities in the metabolism of plants grown without iron. That plants grown for 40 days in the iron-free solution made only slightly more growth than those grown for 13 days, although they contained much more iron, is probably due in part at least to the immobility of iron in the plant (7). Considering the extremely low concentration from which plants absorb their iron in solutions supplied with iron, it is probable that the plants obtained traces of iron even from the "iron-free" solutions, as it would be very difficult to exclude one part of iron in several hundred million of solution.[1]

The attempt to determine the soluble iron in the various solutions was unsuccessful because of the impossibility of distinguishing between col-

[1] If the difference between the iron present in plants grown 13 days and 40 days (Table XV) is due to iron absorbed from the solution, 0.0000055 gm. of ferric oxid (Fe_2O_3) was absorbed between the thirteenth and fortieth day by the three plants grown in each flask. As 1,600 c. c. of solution were supplied per flask during this interval, the concentration of soluble iron, expressed as Fe_2O_3, would have been less than 3½ parts in 1,000,000,000.

loidal and soluble iron at relatively great dilutions. From the amount of iron supplied and the amount absorbed it appeared that there could have been not more than approximately 1 part in 10,000,000 in solution at one time and very probably much less. The much larger quantities of iron found in the filtrate of the nutrient solutions were attributed to colloidal iron, which a previous test showed to be unavailable to the plant.

DISCUSSION OF RESULTS

The reactions of the solutions used in this work could not be exactly measured by titration because of the presence of interfering ions. It is evident, however, that the acid solution was relatively quite strongly acid as it contained only monopotassium phosphate besides a trace of sulphuric acid, the neutral solution was nearly neutral as it contained a mixture of mono and di-potassium phosphates, and the alkaline solution was slightly alkaline from the presence of calcium carbonate and its reaction with the phosphates.

The preceding tests demonstrated that the growth of rice was markedly dependent on the quantity and form of iron added to these nutrient solutions and apparently dependent on the reaction of the solution only so far as it affected the availability of the iron. These facts are important in bearing on the nature and cause of lime-induced chlorosis, rice being markedly affected with this nutritional disturbance. Previous work in soil cultures showed pretty decisively that lime-induced chlorosis was not caused by lack of any mineral nutrient except possibly iron, but did not show whether the reaction of the soil in itself affected the plants. The present study seems to substantiate the previous work and show, moreover, that rice is not particularly sensitive to the reaction *per se*, provided the iron supply is maintained. While in soils there are many more factors affecting the availability of iron than in nutrient solutions, the preceding results point strongly to calcium carbonate diminishing the quantity of available iron in a soil through affecting the reaction. Direct evidence on this latter point is afforded by the results of Morse and Curry (11) and Ruprecht and Morse (12). The results also point to different iron compounds, particularly the organic and inorganic compounds, varying greatly in their availability in calcareous soils. The existence of organic iron compounds in the soil has been pointed out by Hartwell and Kellogg (8).

While all the tests reported here were carried out with rice, certain results are of general interest in relation to the proper composition of plant-nutrient solutions. The extent to which growth was dependent on the iron supply in the previous tests shows how important it may be to consider the form and quantity of iron used in the nutrient solution. Evidently the addition of a few drops of a dilute iron solution, as recommended in most plant physiologies, may not insure an adequate supply of iron. While the color of the leaves will indicate a marked deficiency of iron, a slight deficiency may materially diminish the yield without mate-

rially affecting the appearance of the plants. It is, therefore, not sufficient to judge the adequacy of the iron supply by the mere color of the leaves. Also a sufficiency of iron can not be insured by simply increasing the quantity of iron added to the solution as this may even diminish the amount of available iron.

Although this work demonstrated chiefly the reaction of the solution and the source of iron as factors influencing the availability of iron, the importance of other conditions was indicated. Undoubtedly the frequency with which the nutrient solution is changed influences appreciably the availability of the iron. The results in Table XII showed that the precipitated iron increased with the age of the solution and also with the growth of plants in the solution. This increase in precipitated iron probably accompanied a certain decrease in soluble iron, as there was doubtless some balance between the precipitated, colloidal, and soluble iron.

Doubtless absorption also affected the quantity of soluble iron in certain cases. The diminution in growth following the addition of carbon black to the solution with 0.002 gm. of iron per liter in experiment 10 was probably caused by a decrease in soluble iron through simple adsorption. In experiment 4 the decrease in available iron following an increase in the quantity of iron added to the alkaline solution may have been partially due to the adsorption of soluble iron by the larger precipitate of iron.

On the basis that hydrolysis was the chief factor determining the amount of soluble iron in the solutions, one would not expect that increasing the phosphates in the neutral solution would appreciably affect the iron assimilated by the plants. Experiment 11 confirmed this.[1]

This work furnished no evidence of rice being able to assimilate other than soluble iron, but tended to confirm previous work showing that even colloidal iron is unavailable (6). It did show, however, that rice used iron, which must have been present in exceedingly low concentrations.

The facts established concerning the availability of iron in these nutrient solutions help explain results obtained by certain investigators with other nutrient solutions. It is realized, however, that the availability of iron in each nutrient solution probably varies according to the concentration and composition of the solution as well as according to the method of conducting the cultural test.

The chlorosis of peas in certain nutrient solutions observed by Mazé et al. (10) was evidently not due to the excretion of calcium carbonate by the roots, but to salts in the solution depressing the availability of the iron. Probably the potassium silicate, which is strongly hydrolyzed into potassium hydroxid, was chiefly responsible for the nonavailability of the iron. The chlorosis observed by Von Crone (3) with plants in certain nutrient solutions was not due to the soluble phosphates, but probably to a deficiency of iron. In certain solutions the lack of iron was

[1] If the concentration of phosphate ions had been very low, such as would be afforded by tricalcium or ferric phosphate, doubtless the addition of soluble phosphates would have depressed the available iron.

doubtless due to the reaction of the solution, calcium carbonate being present. In other solutions where ferric phosphate was the source of iron, addition of soluble phosphates produced chlorosis. The soluble phosphates here evidently precipitated the very small amount of iron that went into solution from the decomposition of ferric phosphate. Von Crone made the mistake of assuming that because ferric or ferrous phosphate furnished sufficient iron in some solutions that it did in all solutions. Doubtless the reason that ferrous phosphate furnished sufficient iron in Von Crone's solution was due to the fact that the concentration of phosphate ions was also particularly low.[1]

The results of this work, as well as that of Takeuchi (15) and Benecke (1), showed that soluble phosphates do not in themselves produce chlorosis. Benecke in his criticism of Von Crone's solution failed to take into account that part of the iron he determined in his tests of the solubility of iron phosphates was colloidal iron.

SUMMARY

Rice was grown in acid, neutral, and alkaline solutions with different forms and quantities of iron to determine whether rice is particularly sensitive to the reaction of the solution and whether the reaction of the solution influences the assimilation of iron.

In nearly all cases growth was much better in the nutrient solutions employed with 0.008 gm. of iron per liter than with 0.002 gm. When judged by the growth of plants ferrous sulphate, ferric citrate, and ferric tartrate afforded sufficient iron when used in proper quantities in the acid and neutral solutions. Ferric chlorid was an inferior source of iron, and dialyzed iron utterly inadequate. Only ferric tartrate furnished sufficient iron in the alkaline solution.

Plants grown in the acid solutions contained the highest percentages of iron. Plants grown in the neutral solutions contained higher percentages of iron than those grown in the alkaline solutions when some forms of iron were used, but equal percentages when other forms of iron were used. The percentages of nitrogen, phosphoric acid, lime, magnesia, and carbon-free ash in plants grown in six different solutions did not vary appreciably when compared with the iron content.

It was evident that rice was not particularly sensitive to the reaction of the solution, except as the reaction influenced the availability of the iron. This substantiates previous work in showing that lime-induced chlorosis is caused by a lack of iron and indicates strongly that the only action of carbonate of lime in inducing chlorosis lies in diminishing the availability of the iron.

The amount of available iron in the different solutions could not be determined analytically, because of the impossibility of distinguishing

[1] Ferrous and ferric phosphate evidently afford iron and phosphoric acid not through the dissolving action of plant roots, as Crone believed, but through the hydrolytic decomposition of these compounds. As a result of this decomposition, colloidal iron hydroxid is formed, as well as phosphate ions and a very small amount of soluble iron (9, 2).

between colloidal and soluble iron. Calculations showed, however, that the concentration of available iron in many cases must have been less than one part in 10,000,000 of solution

Reference was made to the bearing of these results on the proper composition of plant nutrient solutions.

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INFLUENCE OF BORDEAUX MIXTURE ON THE RATES OF TRANSPIRATION FROM ABSCISED LEAVES AND FROM POTTED PLANTS

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INTRODUCTION

Since the introduction of Bordeaux mixture much work has been done on the effects which this fungicide has on the growth of normal plants. For more than 20 years this work has occupied the attention of pathologists. During this time progress has been made, but there still remains much to be done. Many observations have been made as to the effect which an application of this spray has upon transpiration rates, and a review of the literature brings out the fact that the conclusions drawn from these observations are conflicting.

Rumm (15)[2] found that when abscised leaves were placed in water the unsprayed ones wilted first; from this he concluded that there is a decrease in the rate of transpiration following an application of the spray. Clinton (4) expressed the view that the water pores and stomata of potato leaves are clogged by the spray and as a result transpiration is decreased. Schander (16), Bayer (2), and Müller-Thurgau (14) each expressed the opinion that decreased transpiration rates follow spraying with Bordeaux mixture.

As a result of extensive investigations, Frank and Kruger (8, 9) conclude that the water loss from sprayed plants is greater than from plants not so treated. Bain (1) likewise found that an increased rate of transpiration occurs in peach seedlings as a result of spraying. He arrived at this conclusion from the fact that he found it necessary to supply water more frequently to the roots of seedlings that had been sprayed. More recently, however, Duggar and Cooley (6, 7) have furnished direct evidence bearing upon this question. In a series of very carefully performed experiments they have demonstrated that not only does a film of Bordeaux mixture on the leaves of castor beans, tomatoes, and potatoes increase their rates of transpiration but that other surface films have a similar effect. They have brought out the fact that certain specific qualities of the films applied are definitely related to the phenomenon of increased transpiration. They further state that the color of the film applied is also a factor to be considered in this connection.

[1] Thanks are due to Dr. M. T. Cook for suggesting the problem and to Dr. J. W. Shive for valuable suggestions during the progress of the work and for aid and criticism in the preparation of the manuscript.

[2] Reference is made by number to "Literature cited," p. 547–548.

While the last-named authors have definitely established the fact that surface films with certain specific characteristics have an accelerating influence on rates of transpiration when applied to the leaves of castor bean, tomato, and potato plants, the problem here involved seemed to be of sufficient importance to warrant further investigation in a different locality and under different conditions.

It was the purpose of the experiments here reported to determine the influence of Bordeaux mixture on the rates of transpiration of abscised leaves of several species, as well as to determine the effect of this spray material upon the rates of water loss from a variety of potted plants. The experiments were carried out in the greenhouse of the Department of Plant Pathology of the New Jersey Agricultural Experiment Station.

EXPERIMENTS WITH ABSCISED LEAVES

EXPERIMENTAL METHODS.—Abscised leaves of radish (*Raphanus sativus* L.), bean (*Phaseolus vulgaris* L.), Swiss chard (*Beta cycla* L.), *Hibiscus cardinalis*, *Clerodendrum balfouri*, *Caladium* sp., *Datura meteloides*, and castor bean (*Ricinus communis* L.) were used. The leaves, together with portions of the stems, were severed from the plants and the cut ends immediately placed in water. They were then taken to the greenhouse room, where the experiments were carried out. The leaves were now cut off under water and the petioles were inserted into Erlenmeyer flasks having a capacity of 180 c. c.; the flasks were nearly filled with water. A layer of cotton was then placed tightly around the leaf petiole just at the surface of the water; this served to hold the leaf in place. The flasks were sealed by pouring melted wax over the cotton around the leaf petioles. This wax was prepared according to the formula of Briggs and Shantz (3) and consisted of a mixture of about 80 per cent of paraffin and 20 per cent of petrolatum. The mixture had a melting point of about 45° C. By pouring a layer of this wax about 1 cm. thick on the layer of cotton around the leaf petioles the leaves were held firmly in place. To permit the entrance of air into the flasks as water was removed by transpiration from the leaf a small hole was made in the wax with a pin.

In testing the effect of Bordeaux mixture on the rates of transpiration of abscised leaves, six leaves of each species were employed. These were chosen from larger groups of leaves which had been mounted as above described and allowed to stand for some time in order to become adjusted to the new conditions. Leaves of the different species used were chosen with special reference to equality of surface exposed and also with reference to similarity of general appearance. The six leaves chosen were divided into two groups of three each. For facility in comparison and for ease of reference in discussion, one of these groups will be designated "series A" for periods before treatment and "series A'" for periods after treatment; the other group, remaining untreated throughout the experiment, will be designated "series B."

It is, of course, not possible in experiments of this nature to subject each leaf to precisely the same changes in aerial conditions. Special precautions were taken, however, to arrange the leaves involved in a single experiment in such a manner with reference to each other and to their surroundings that each might experience, as nearly as possible, the same changes in environmental conditions. This was accomplished by placing the flasks in rows 2 feet apart on a table centrally located in the greenhouse, where air currents from ventilators or open doors would affect the plants similarly. The arrangement decided upon for each experiment was maintained until the experiment was terminated. The leaves were removed from their positions only for the purpose of weighing, and each leaf was returned to the position previously occupied as soon as the weighing was completed.

Each flask with its leaf (in series A and series B) was weighed to 0.01 gm., allowed to stand for a definite time period, and again weighed. The difference between the two readings, of course, gave the absolute transpiration for the time period. The first period of exposure, before spraying the leaves of series A with Bordeaux mixture, may be regarded as the standardization period, and the leaves of the control series B may be regarded as the standard leaves for comparison. At the close of the standardization period the leaves of series A, which then became series A′, were sprayed with Bordeaux mixture. This spray was prepared in the usual way and contained 12 gm. of copper sulphate and 12 gm. of lime in 1 liter of the mixture, approximating the 5–5–50 formula of agricultural practice. Both upper and lower surfaces of the leaves were sprayed, the spray being applied with an atomizer. This method yielded a very uniform film of spray over the leaf surfaces.

A comparison of the ratios between the transpiration quantities of series A and those of series B for the time period before spraying with similar ratios for the time period after spraying will determine whether the transpiration rates of the leaves for the periods after spraying have changed. For the sake of convenience in the treatment of ratios, transpiration quantities for series A will be termed "A" for periods before treatment and "A′" for periods after treatment. The corresponding transpiration quantities for series B will be designated "B" throughout, since the leaves of series B were not treated with Bordeaux mixture. If then the ratio of A to B is greater than the ratio of A′ to B, it follows that the rates of transpiration of the sprayed leaves have suffered a decrease relative to the rates of the unsprayed leaves. If, however, the ratio of A to B is less than the ratio of A′ to B, then the rates of transpiration of the sprayed leaves have increased, relative to the rates of the unsprayed leaves. In making such comparisons it must be assumed, of course, that the ratio of A to B for successive time periods would remain unchanged if the leaves of series A were not treated. It could scarcely be expected that the ratio of A to B would remain constant for successive intervals, since it is not possible to subject all the leaves to precisely the same changes in

aerial conditions. Furthermore, internal changes continually taking place in the leaves would tend to cause some variations in the ratio of A to B, since the degree of these changes would certainly vary from leaf to leaf, even supposing the nature of these changes to be the same in all the leaves. It may reasonably be supposed, however, that variations in the ratio of A to B for successive intervals, due to internal changes and to differences in the changes of environmental conditions experienced by the different leaves of a series, are comparatively small. This was, indeed, found to be the case in a series of preliminary experiments. The time period during which an experiment may be conducted with relatively very small variations in the ratio of A to B, owing to conditions other than the treatment with spray, varies, of course, with the different species.

EXPERIMENTAL RESULTS.—The experimental data showing the effect of Bordeaux mixture on the transpiration of abscised leaves are presented in Table I. The first column of this table gives the names of the various species dealt with and the time at the beginning and end of each experimental period. This is followed, under "Periods before treatment," by three columns giving transpiration quantities, in grams, of the three leaves of series A; then are given three columns presenting transpiration data for series B for the same time periods. The last column of this section gives ratios obtained by summing the values of the transpiration quantities of the three leaves (on the same horizontal line) of series A and dividing this summed value by the summed value of the corresponding transpiration quantities for series B. The second section, under "Periods after treatment," presents in the same way as the first the data comparing series A′ with series B. The table is further divided into a number of horizontal sections, each section presenting all of the data for a single species.

The data included in the first horizontal section of Table I represent an experiment extending over a total time period of more than five days. The transpiration data for datura leaves presented in the second horizontal section of the table represent an experiment extending over a total time period of more than four days. During these two experiments weighings were made each day, as indicated. The data presented in the remaining sections of the table represent experiments conducted mainly to determine whether the spray becomes effective in its influence on transpiration, immediately after drying on the leaves, or whether modified rates of water loss begin at some later period. All of these experiments were conducted during the same day, extending over a total time period of a little more than 10 hours. Water loss from each leaf was determined at intervals of two hours. The containers used in these experiments were small, so that weighings could be made to 0.01 gm. At the end of the two standardization periods the leaves of the A series were sprayed on the upper and lower foliar surfaces, and weighings were made soon after the spray had completely dried on the leaves.

TABLE I.—*Effect of Bordeaux mixture on the rates of transpiration of abscised leaves for periods before and after treatment*

PERIODS BEFORE TREATMENT

Plant and period.	Transpiration quantities.						Ratio A : B.
	Series A.			Series B (control).			
	Leaf 1.	Leaf 2.	Leaf 3.	Leaf 1.	Leaf 2.	Leaf 3.	
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	
Ricinus communis:							
Feb. 1, 8.20 a. m.	6.40	3.60	2.60	1.30	4.50	6.20	1.05
1, 3.30 p. m.							
2, 10.00 a. m.	1.40	2.40	4.00	2.70	2.70	4.60	.78
2, 6.00 p. m.							
3, 8.30 a. m.	1.70	4.20	3.50	2.80	4.80	5.90	.70
3, 4.00 p. m.							
Total	9.50	10.20	10.10	6.80	12.00	16.70	.84
Datura metaloides:							
Mar. 16, 4.00 p. m.	10.90	8.20	1.50	9.50	5.80	7.90	.89
17, 1.25 p. m.							
17, 1.25 p. m.	4.70	4.20	2.20	4.80	4.10	4.60	.85
18, 11.30 a. m.							
Total	15.60	12.40	3.70	14.30	9.90	12.50	.86
Phaseolus vulgaris:							
Mar. 22, 8.50 a. m.	.08	.14	.18	.08	.43	.21	.56
22, 10.40 a. m.							
22, 10.40 a. m.	.07	.14	.21	.13	.18	.20	.82
22, 12.40 p. m.							
Total	.15	.28	.39	.21	.61	.41	.67
Beta cycla:							
Mar. 22, 9.45 a. m.	.17	.27	.23	.23	.45	.25	.72
22, 10.35 a. m.							
22, 10.35 a. m.	.07	.20	.21	.19	.31	.27	.62
22, 1.35 p. m.							
Total	.24	.47	.44	.42	.76	.52	.68
Raphanus sativus:							
Mar. 22, 9.10 a. m.	.17	.25	.....	.10	.30	.17	.72
22, 11.10 a. m.							
22, 11.10 a. m.	.16	.33	.....	.21	.27	.20	.72
22, 12.10 p. m.							
Total	.33	.58	.....	.31	.57	.37	.72
Caladium sp. (small variety):							
Mar. 22, 9.05 a. m.	.30	.28	.30	.33	.29	.21	1.06
22, 10.55 a. m.							
22, 10.55 a. m.	.25	.20	.25	.20	.20	.14	1.29
22, 12.55 p. m.							
Total	.55	.48	.55	.53	.49	.35	1.15
Hibiscus cardinalis:							
Mar. 22, 9.30 a. m.	.04	.07	.08	.12	.10	.05	.73
22, 11.20 a. m.							
22, 11.20 a. m.	.09	.08	.05	.05	.18	.10	.67
22, 1.25 p. m.							
Total	.13	.15	.13	.17	.28	.15	.68
Clerodendron balfouri:							
Mar. 22, 8.35 a. m.	.09	.13	.10	.13	.10	.07	1.06
22, 10.30 a. m.							
22, 10.30 a. m.	.11	.15	.13	.15	.10	.14	1.00
22, 12.30 p. m.							
Total	.20	.28	.23	.28	.20	.21	1.02
Datura metaloides:							
Mar. 22, 8.15 a. m.	.66	.48	.92	1.03	.27	.44	1.18
22, 10.15 a. m.							
22, 10.15 a. m.	.45	.48	1.09	.75	.25	.39	1.45
22, 12.10 p. m							
Total	1.11	.96	2.01	1.78	.52	.80	1.31

TABLE I.—*Effect of Bordeaux mixture on the rates of transpiration of abscised leaves for periods before and after treatment*—Continued

PERIODS AFTER TREATMENT

Plant and period.	Transpiration quantities.						Ratio A′ : B.
	Series A′.			Series B (control).			
	Leaf 1.	Leaf 2.	Leaf 3.	Leaf 1.	Leaf 2.	Leaf 3.	
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	
Ricinus communis:							
Feb. 4, 9.30 a. m.	3.50	5.50	4.70	3.00	2.30	4.10	1.45
4, 3.30 p. m.							
5, 8.30 a. m.	3.60	3.40	4.40	3.10	2.80	4.00	1.15
5, 4.30 p. m.							
6, 12.00 a. m.	2.30	1.30	2.40	1.90	1.70	2.30	1.01
6, 5.30 p. m.							
Total	9.40	10.20	11.50	8.00	6.80	10.40	1.23
Datura metaloides:							
Mar. 18, 4.00 p. m.	5.00	7.10	3.00	3.10	1.30	2.70	2.12
19, 9.00 a. m.							
19, 9.00 a. m.	3.30	4.90	5.00	1.30	1.30	6.00	1.53
20, 4.00 p. m.							
Total	8.30	12.00	8.00	4.40	2.60	8.70	1.82
Phaseolus vulgaris:							
Mar. 22, 3.00 p. m.	.13	.24	.19	.11	.06	.14	1.80
22, 4.50 p. m.							
22, 4.50 p. m.	.09	.16	.12	.09	.10	.08	1.37
22, 6.50 p. m.							
Total	.22	.40	.31	.20	.16	.22	1.60
Beta cycla:							
Mar. 22, 3.55 p. m.	.19	.19	.22	.19	.22	.15	1.07
22, 5.55 p. m.							
22, 5.55 p. m.	.11	.17	.16	.12	.15	.14	1.07
22, 7.55 p. m.							
Total	.30	.36	.38	.31	.37	.29	1.07
Raphanus sativus:							
Mar. 22, 3.40 p. m.	.32	.52	.....	.19	.23	.21	1.33
22, 5.35 p. m.							
22, 5.25 p. m.	.21	.38	.....	.13	.20	.26	1.00
22, 7.35 p. m.							
Total	.53	.90	.....	.32	.43	.47	1.17
Caladium sp. (small variety):							
Mar. 22, 3.30 p. m.	.34	.10	.34	.06	.15	.02	3.39
22, 5.20 p. m.							
22, 5.20 p. m.	.09	.14	.16	.10	.13	.10	1.18
22, 7.25 p. m.							
Total	.43	.24	.50	.16	.28	.12	2.08
Hibiscus cardinalis:							
Mar. 22, 3.45 p. m.	.14	.14	.14	.05	.07	.04	2.62
22, 5.45 p. m.							
22, 5.45 p. m.	.09	.12	.18	.06	.06	.04	2.42
22, 7.40 p. m.							
Total	.23	.26	.32	.11	.13	.08	2.53
Clerodendron balfouri:							
Mar. 22, 3.10 p. m.	.08	.12	.12	.10	.09	.07	1.23
22, 5.05 p. m.							
22, 5.05 p. m.	.09	.10	.10	.06	.05	.04	1.70
22, 7.00 p. m.							
Total	.17	.22	.22	.18	.14	.11	1.46
Datura metaloides:							
Mar. 22, 3.15 p. m.	.72	.81	.18	.53	.28	.35	1.47
22, 5.15 p. m.							
22, 5.15 p. m.	.52	.59	.79	.37	.25	.22	2.26
22, 7.10 p. m.							
Total	1.24	1.40	.97	.90	.53	.57	1.80

A study of the last column of Table I brings out the fact that the first 2-hour period after spraying yielded higher ratio values than the second period after spraying. A notable exception to this, however, is the experiment with *Datura metaloides* conducted on March 22. Here the second period after spraying yields a ratio value considerably higher than the first, while in the experiment with Swiss chard the ratios for the two periods after spraying have the same value. The experiments with Datura sp. and with castor-bean leaves, extending over time periods of four days and five days, respectively, show a decrease in the ratio values for the time periods of successive days. It appears, therefore, that in these experiments the highest rate of water loss from the leaves due solely to the influence of the spray occurred soon after the spray had dried upon the leaves, certainly within the first 2-hour period, after which there was a gradual decrease in the transpiration rates as influenced by the surface film of the spray. As is clearly brought out in the experiment with castor-bean leaves, this gradual decrease in the rates of water loss from the leaves, as indicated by the ratio values, extends through a period of three days after spraying. The gradual decrease in the ratio values during the period after spraying is undoubtedly caused by the gradual disintegration and wearing away of the surface films. It may therefore be assumed that whatever may be the physical basis for the increased evaporation rates from plant surfaces following the application of Bordeaux mixture, a modification of leaf structure in response to a chemical stimulus induced by the spray can not be responsible for the increased transpiration rates.

In Table II is given a brief summary of the average data presented in Table I. The ratio values of total transpiration quantities for standardization periods are here considered as 1.00, while the corresponding ratio values for periods after spraying are expressed in terms of these.

TABLE II.—*Summary of the average data from Table I. Ratio values of A′ to B for periods after spraying are relative to the ratio values of A to B for standardization periods expressed as 1.00*

Plant.	Duration of experiment.	Transpiration (ratio A′:B).
Ricinus communis	6 days	1.50
Datura metaloides	4 days	2.12
Phaseolus vulgaris	10 hours	2.39
Beta cycla	do	1.57
Raphanus sativus	do	1.63
Caladium sp.	do	1.81
Hibiscus cardinalis	do	3.72
Clerodendron balfouri	do	1.43
Datura metaloides	11 hours	1.37
Average		1.99

From the brief summary in Table II it will be observed that the lowest ratio value for any period after treatment is 1.37 times the ratio value for the corresponding standardization period, while the highest ratio value is 3.72 times the ratio value for the corresponding standardization period. The average value of the ratios for periods after spraying is 1.99 times the average ratio for the standardization periods. Expressed in another way, the leaves sprayed with Bordeaux mixture showed an increase in the rates of water loss, relative to their respective controls in each case, varying from 37 per cent for the lowest increase to 272 per cent for the highest. The average rate of water loss by transpiration of the species here employed shows an increase of 99 per cent over the average rate for the standardization period. The degree of this accelerating influence of Bordeaux mixture on the rates of transpiration varies considerably with the different species of leaves, as is indicated by this wide range of variation in the ratio values for the different leaves.

The leaves employed in these experiments were allowed to remain in the places occupied during the experiments for some time after the experiments had been terminated and were kept under observation to determine whether the sprayed or the unsprayed leaves should first show signs of wilting. It was observed that in every instance the sprayed leaves showed signs of wilting at some period preceding the time at which the unsprayed leaves of the same species began to wilt. This is only what would be expected in view of the fact that the average rate of water loss from the sprayed leaves is nearly double that from the unsprayed leaves during the experimental time period.

EXPERIMENTS WITH POTTED PLANTS

EXPERIMENTAL METHODS.—In the experiments with potted plants the method of procedure was similar to that followed in the experiments with abscised leaves. The plants employed consisted of tomato (*Lycopersicon esculentum* Mill.), cabbage (*Brassica oleracea* L.), pepper (*Capsicum annuum* L.), egg plant (*Solanum melongena* L.), and soy bean (*Glycine hispida* M.). These plants, excepting the soy beans, were grown in beds of soil in the greenhouse until they had attained a size suitable for experimentation of this character. They were then transplanted to earthenware pots glazed inside and outside and having a capacity of approximately 1.5 liters. In order to prevent evaporation from the surface of the soil, melted wax prepared according to the Briggs and Shantz formula was poured over the surface of the soil in each pot, thus making a perfect seal.

Water was automatically supplied to the roots of the plants by means of the autoirrigator. This instrument has been described by Livingston (12) and later by Hawkins (11). It consists of a porous clay cup closed by a rubber stopper through which extend two glass tubes. One of these tubes is bent into the form of an inverted U having one arm considerably

longer than the other. The short arm extends through the rubber stopper into the cup. The bend of the U turns over the edge of the container and the end of the longer arm dips into a reservoir of water below. The second glass tube passes through the rubber stopper and ends just at its lower surface. To the upper end of this glass tube a short piece of rubber tubing is attached. This is closed by means of a pinchcock or screw clamp when the instrument is installed. In order to install the instrument, the rubber stopper with its glass tubes is pressed firmly into the mouth of the cup. The cup is then buried in the soil of the container among the roots of the plant. The end of the long arm of the U-tube is dipped into a suitable reservoir of water below. Suction is now applied to the short rubber tube. This causes water to rise through the U-tube into the cup, filling the latter. When the water has risen and filled the cup and the short rubber tube, a pinchcock is applied, thus closing the tube. In installing the instrument, care must be taken that all connections are tight and that all air is removed from the system. As water is removed from the soil by the plant roots, more water passes through the porous walls of the clay cup, replacing that absorbed by the roots. In this way an approximately constant soil-moisture content may be maintained. Any desired moisture content of the soil may be obtained and maintained approximately by simply increasing or decreasing the distance between the surface of the water in the reservoir and the surface of the water in the porous clay cup.

In experiments such as are here reported, a constant moisture content of the soil in which the plants are rooted is of considerable importance, and the old method of supplying water to the roots at stated intervals has proved unsatisfactory.

After installing the autoirrigators and sealing the pots the plants were allowed to stand a week in order to become adjusted to the new conditions before the experiments were begun.

In each experiment 12 plants were employed. These were chosen from a much larger number and were selected with special reference to uniformity of size and vigor. The 12 plants were divided into two groups, each group constituting a series of 6 plants. In order to expose the plants included in a single experiment to the constantly changing aerial conditions in such a way that all might be affected in a somewhat similar manner, they were arranged in two rows, 6 plants to a row, on opposite sides of a greenhouse bench, the rows being placed near the edge of the bench. The bench was centrally located and was not in the direct path of air currents from open doors or ventilators. As a further precaution, the plants were shifted in their positions in the rows each day according to a definite plan previously decided upon.

The plants were weighed each day and the water loss from each plant for the time period immediately preceding was determined. The weighings were made in the order in which the plants were numbered, from 1

to 6, and this plan was maintained throughout the experiment, so that the time periods for each plant between any two weighings were approximately the same. At the end of the standardization period the plants of series A were sprayed. This series now became series A′. The Bordeaux mixture employed was prepared according to the same formula as that used in the experiments with abscised leaves. The spray was applied to the leaves of the plants with an atomizer. This method gives a very uniform film of the spray over the leaf surfaces. The spray was applied to both the upper and lower surfaces of the leaves, except in the experiments with cabbage plants.

At the close of an experiment the tops were severed from the roots just at the surface of the wax seal, and the green weights were immediately obtained. The plants were then placed in weighing bottles and dried in an oven at a temperature of from 76° to 100° C. for a period of 28 hours, after which they were dried to constant weight at a temperature of from 102° to 105° C. The bottles were then transferred to a large desiccator and were allowed to cool to room temperature before weighing.

Measurements were taken of the evaporating power of the air in the greenhouse room where the experiments were carried out. These measurements were made by means of standardized porous-cup atmometers (13); the instruments were placed among the plants on the greenhouse bench and readings were taken each day at the time when the plants were weighed. The readings were corrected to the Livingston cylindrical standard by multiplying by the coefficient of correction of the cup used. In the time during which the experiments were conducted the water loss from the porous-cup atmometer gave a daily mean of 10.4 c. c.; a maximum daily rate of 24.0 c. c. (on March 1) and a minimum daily rate of 6.0 c. c. (on March 6).

As in the experiments with abscised leaves, one of the two groups of plants comprised in a single experiment will be designated "series A." The other group, remaining untreated throughout the experiment, will be designated "series B." In the experiments with potted plants transpiration quantities for series A and for series B will be denoted "A" and "B" in their respective series. This method of notation is precisely the same as that adopted in the experiments with abscised leaves.

In presenting the data for the experiments with potted plants the ratios between the water loss per gram of green substance for periods before and after spraying, as well as the corresponding water loss per gram of dry substance, will also be treated. Green- and dry-weight values could, of course, not be obtained until the close of an experiment. These values must serve, therefore, for the calculation of ratios for the standardization periods as well as for the periods after spraying. This, however, could make no material difference in the ratio values for periods before spraying, since any increase in the weight of plant substance due

to the growth of the plants during the periods after spraying would affect approximately alike the green- and dry-weight values of both series A′ and series B. If, in the treatment of these ratios the green-weight values for series A and for series A′ (the values for these two series being the same) are denoted by "G," and the corresponding values for series B are denoted by "G′," it follows that the ratios of A to G, A′ to G, and B to G′ must represent the water loss per gram of green substance for series A, series A′, and series B, respectively. Now, the ratio between the average water loss per gram of green substance for series A and for series B (periods before spraying) is expressed by $\frac{A:G}{B:G'}$, or $\frac{AG'}{BG}$. The corresponding ratio value between the average water loss per gram of green substance for series A′ and for series B (periods after spraying) is expressed by $\frac{A':G}{B:G'}$, or $\frac{A'G'}{BG}$. If, therefore, the ratio of AG′ to BG is less than that of A′G′ to BG, the water loss per gram of green substance must be greater for the sprayed plants than for the unsprayed.

The dry-weight values for series A′ and for series B will be denoted by "D" and "D′," respectively, and the ratios between the water loss per gram of dry substance for periods before spraying and the corresponding ratio for periods after spraying, derived in the same manner as the ratios between the water loss per gram of green substance, are expressed by the ratios of AD′ to BD and A′D′ to BD, for standardization periods and for periods after spraying, respectively.

CABBAGE PLANTS

In this experiment the plants used were vigorous and fairly uniform in size. The standardization period continued from March 1 to March 4. The period after spraying began on March 4 and continued to March 17.

Considerable difficulty was encountered in attempts to spray the leaves of the cabbage plants. The bloom on the surface of the leaves prevented the spray material from adhering. Various substances were added to the spray material in an attempt to find something which would cause the spray to adhere to the leaves. Rosin-fish-oil soap in the proportion of 2 pounds to 50 gallons of Bordeaux mixture, as recommended by Hawkins (10), proved to be the most effective; but even this did not give the desired results. It was found that by lightly brushing the surface of the leaves with a wad of dry absorbent cotton, and by immediately afterwards applying the spray, a surface film of the spray could be obtained. This film was by no means ideal, but it was better than that secured by using the rosin-fish-oil soap in connection with the Bordeaux mixture. The leaves of the control series B were also lightly brushed with a wad of absorbent cotton at the same time that the leaves of series A′ were thus treated. Only the upper surfaces of the leaves were thus treated and sprayed.

In Table III are given the data for the experiment with potted cabbage plants. This table presents first in a horizontal section the summed transpiration quantities for each plant and the average for the plants in each series. It also gives the green and dry weight values for each plant and the average for the plants in series A′ and in series B. This section is followed below and in the order here given by (1) the ratios between the average transpiration quantities of the two series for the periods before and after spraying; (2) the ratios between the quantities representing the average water loss per gram of green substance, of the two series in periods before and after spraying; and (3) the ratio between quantities representing the average water loss per gram of dry substance, of the two series in the periods before and after spraying. The difference between the two ratios in a pair, on the same horizontal line, is given to the right. The plus (+) sign following the difference indicates that the ratio for the period after spraying is higher than the corresponding ratio for the standardization period.

TABLE III.—*Effect of Bordeaux mixture on the rate of transpiration of potted cabbage plants*

[Period before treatment, Mar. 1 to Mar. 3; period after treatment, Mar. 4 to Mar. 17, 1916]

Plant No.	Transpiration quantities.				Green weight of tops.		Dry weight of tops.	
	Period before treatment.		Period after treatment.					
	Series A.	Series B.	Series A′.	Series B.	Series A′.	Series B.	Series A′.	Series B.
	(*A*)	(*B*)	(*A′*)	(*B*)	(*G*)	(*G′*)	(*D*)	(*D′*)
1	67.6	33.4	1015.2	342.1	56.48	36.92	10.48	6.15
2	46.5	32.7	709.0	523.9	58.28	39.18	8.98	7.70
3	71.1	36.1	1041.9	493.3	58.81	40.72	10.90	6.20
4	42.6	30.7	642.4	585.5	42.82	38.11	7.74	6.99
5	60.5	33.4	1008.8	685.5	69.90	57.46	9.92	10.04
6	32.2	38.3	516.8	497.9	42.11	49.27	9.11	8.76
Average	53.4	34.1	835.7	521.4	54.72	43.61	9.36	7.64
Transpiration ratio	A: B=1.56		A′: B=1.60		Difference			0.04+
Water loss per gram of green weight ratio	AG′: BG=1.24		A′G′: BG=1.28		Difference			.04+
Water loss per gram of dry weight ratio	AD′: BD=1.27		A′D′: BD=1.31		Difference			.04+

It will be observed from Table III that the ratio values for the period after treatment are, in each case, slightly higher than the corresponding ratio values for the period before treatment. This indicates that the average rate of water loss by transpiration from the sprayed plants (series A′) was slightly higher, relative to the average rates from the control plants, than was the corresponding ratio of water loss from those same plants (series A) for the period before treatment. It is to be noted, however, that while the ratios between average quantities for the period after spraying are all higher than the corresponding ratios for the period before treatment, the individual ratios for the period after treatment

vary on either side of the average ratios for the standardization period. In this experiment with cabbage plants it may not, therefore, be stated with entire certainty that an application of Bordeaux mixture has an accelerating influence on the rates of water loss, since the difference between the ratio values for the periods before and after treatment are no greater than might be expected to lie within the limits of experimental error. On the other hand, the spray was applied only to the upper surfaces of the leaves, so that only one-half of the transpiring surface was affected by the spray material. Furthermore, stomatal transpiration from the upper surfaces of the leaves should be somewhat less than from the lower surfaces, considering the number of stomata per square millimeter on the upper leaf surface to be 219 and that on the lower surface to be 301, according to Duggar (5, p. 91). This would still further tend to minimize any accelerating influence which the spray might have on the rates of water loss.

PEPPER PLANTS

The plants used in the experiment with peppers were very uniform in size, about 8 inches tall, vigorous, and were just beginning to bloom when the experiment was begun. The experiment continued during a time period of 16 days. The standardization period extended from February 26 to March 3; the period after treatment continued from March 3 to March 14. The results of this experiment are given in Table IV. The arrangement of this table and subsequent ones is precisely the same as that employed in the presentation of the data for the experiment with cabbage plants.

TABLE IV.—*Effect of Bordeaux mixture on the rate of transpiration of potted pepper plants*

[Period before treatment, Feb. 26 to Mar. 3; period after treatment, Mar. 3 to Mar. 14, 1916]

Plant No.	Transpiration quantities.				Green weight of tops.		Dry weight of tops.	
	Period before treatment.		Period after treatment.					
	Series A.	Series B.	Series A′.	Series B.	Series A′.	Series B.	Series A′.	Series B.
	(*A*)	(*B*)	(*A′*)	(*B*)	(*G*)	(*G′*)	(*D*)	(*D′*)
1	38.1	35.7	109.3	43.0	3.70	5.02	0.75	0.83
2	37.9	17.0	91.4	37.7	2.08	2.57	.45	.31
3	31.4	27.9	63.3	42.5	4.57	2.25	.70	.40
4	37.3	40.0	102.8	103.3	4.40	4.87	.65	.73
5	29.8	32.9	65.2	54.7	4.20	4.73	.69	.78
6	57.9	20.9	114.8	37.9	6.80	2.75	1.12	.47
Average	38.7	29.0	91.1	53.1	4.29	3.69	.72	.58
Transpiration ratio	AB=1.33		A′B=1.71		Difference		0.38+	
Water loss per gram of green substance ratio	AG′ BG=1.14		A′G′ BG=1.47		Difference		.33+	
Water loss per gram of dry substance ratio	AD′ BD=1.07		A′D′BD=1.37		Difference		.30+	

A comparison of the ratio values in Table IV shows very clearly the accelerating influence of a film of Bordeaux mixture on the rates of transpiration of pepper plants. If each of the three ratio values for the period before spraying is made equal to 1.00, the corresponding ratio values for the period after spraying are, in every case, 1.29—that is, 29 per cent higher than the values for the standardization period.

SOY-BEAN PLANTS

The soy-bean plants here employed were about 20 cm. tall when the experiment was begun. Soy-bean seedlings were transplanted directly into the containers when they were about 5 cm. tall. Three seedlings constituted a culture. Four series of cultures were employed, each series comprising six cultures and a total of 18 plants. The four series will be designated series A, series B, series C, and series D, respectively, for the standardization period. Series D is here considered the control series and remained untreated throughout the experiment. The plants of the first three series were treated at the end of the standardization period and are designated "series A′," "series B′," and "series C′," in the order given, for the period after treatment. As in all the experiments here reported, the leaves of the plants of series A′ were covered with a film of Bordeaux mixture. The leaves of series B′ were sprayed with a suspension of barium sulphate in water. The mixture consisted of 28 gm. of barium sulphate in 1 liter of water. This was applied by means of an atomizer in precisely the same manner in which the Bordeaux mixture was applied. The leaves of series C′ were treated with dry copper sulphate in the form of a fine powder. The copper sulphate was prepared by gently heating the crystals in a porcelain crucible until all the water of crystallization had been driven off. After the salt had thus been dried to constant weight, it was ground to a fine powder in a mortar and kept in a desiccator until used. This powder was dusted on the upper surfaces of the leaves until a thin but fairly uniform covering was obtained. During the time period of the experiment the copper sulphate produced no injurious effects upon the leaves, though during this period care was taken to keep the greenhouse room as dry as possible.

For the sake of convenience in presenting the data for this experiment, the transpiration quantities for series A, series B, series C, and series D are denoted by "A," "B," "C," and "D," respectively, for the standardization period; the transpiration quantities for the corresponding series for the period after spraying are denoted by "A′," "B′," "C′," and "D," respectively.

The period of standardization extended from February 29 to March 4; the period after treatment continued from March 4 to March 11. The data for this experiment are presented in Table V.

TABLE V.—*Effect of Bordeaux mixture, copper sulphate, and barium sulphate on the rate of transpiration of potted soy-bean plants*

[Period before treatment, Feb. 29 to Mar. 4: period after treatment, Mar. 4 to Mar. 11, 1916]

Plant No.	Transpiration quantities.							
	Period before treatment.				Period after treatment.			
	Series A (Bordeaux mixture).	Series B (barium sulphate).	Series C (copper sulphate).	Series D (control).	Series A′ (Bordeaux mixture).	Series B′ (barium sulphate).	Series C′ (copper sulphate).	Series D (control).
	(*A*)	(*B*)	(*C*)	(*D*)	(*A′*)	(*B′*)	(*C′*)	(*D*)
1	34.2	53.0	49.0	53.1	128.1	131.3	195.6	177.9
2	64.0	34.0	56.6	58.2	254.7	77.4	198.6	130.2
3	77.5	45.8	39.4	33.5	304.8	103.5	124.9	76.4
4	65.8	38.7	33.5	65.3	217.8	88.7	75.6	142.3
5	67.9	46.5	82.0	46.6	265.1	135.6	252.0	94.8
6	55.6	37.5	92.3	43.9	227.8	87.7	253.2	85.7
Average	60.8	42.5	58.8	50.1	233.0	104.0	183.3	117.8
Transpiration ratio	A D= 1.21	B D= 0.85	C D= 1.17	.....	A′ D= 1.98	B′ D= 0.88	C′ D= 1.55	.....
Difference	.....	.....	.....	.....	0.77+	0.03+	0.38+	.....

From the data of Table V it appears that of the three kinds of materials applied to the leaves of soy-bean plants Bordeaux mixture is the most effective in bringing about an increased rate of water loss. The plants sprayed with Bordeaux mixture here showed, relative to the control plants, an average rate of water loss 63 per cent higher than the average rates of the same plants before spraying. The leaves of the plants dusted with copper sulphate showed a corresponding increase of 37 per cent, while the plants sprayed with barium sulphate show an increase in the average rate of water loss, relative to the control plants, of only 3 per cent over the average rate for the standardization period.

The results here obtained are in entire accord with the conclusions reached by Duggar and Cooley (6) that certain specific characters of the films are important factors, considered in relation to the modified rates of transpiration. The further suggestion of these authors that modified leaf temperature induced by heat absorption due to the color of the surface films is an important factor might also be considered as effective in bringing about the results obtained here, since a film of Bordeaux mixture, being darker in color than either of the two other surface coverings used may be assumed, without any other direct evidence, to have a higher heat-absorbing power than either copper sulphate or barium sulphate. It is scarcely probable, however, that modified leaf temperatures, induced by differences in the heat-absorbing powers of the surface films here used, could account for any great portion of the differences in the rates of water loss observed.

EGGPLANTS

In the experiment with eggplants the standardization period extended two days from April 3 to April 5. The period after spraying continued from April 5 to April 18.

The results of this experiment are presented in Table VI.

TABLE VI.—*Effect of Bordeaux mixture on the rate of transpiration of potted eggplants*

[Period before treatment, Apr. 3 to Apr. 5; period after treatment, Apr. 5 to Apr. 18, 1916]

Plant No.	Transpiration quantities.				Green weight of tops.		Dry weight of tops.	
	Period before treatment.		Period after treatment.					
	Series A.	Series B.	Series A′.	Series B.	Series A′.	Series B.	Series A′.	Series B.
	(*A*)	(*B*)	(*A′*)	(*B*)	(*G*)	(*G′*)	(*D*)	(*D′*)
1	20.9	17.4	400.1	326.2	12.02	11.73	1.67	1.68
2	27.9	23.6	336.2	316.6	11.12	12.63	1.87	1.83
3	23.4	40.0	295.9	426.2	11.35	18.10	1.58	2.72
4	27.4	26.5	445.7	367.0	15.11	14.62	2.14	1.77
5	30.1	33.7	494.1	394.2	19.62	14.19	1.97	1.99
6	18.0	15.9	292.1	334.4	12.40	11.60	1.52	1.55
Average	24.6	26.1	377.3	360.7	13.60	13.81	1.79	1.92
Transpiration ratio	A : B=0.94		A′ : B=1.04		Difference			0.10+
Water loss per gram of green substance ratio	AG′ : BG=0.95		A′G′ : BG=1.06		Difference			.11+
Water loss per gram of dry substance ratio	AD′ : BD=1.00		A′D′ : BD=1.12		Difference			.12+

The results given in Table VI show a pronounced increase in the rate of water loss for the sprayed plants relative to the rates from the control plants, as is indicated by the higher ratio values for the period after spraying. The value of the ratio between transpiration quantities for the period after treatment is here 11 per cent higher than the corresponding ratio value for the period before treatment. Ratios between quantities representing water loss per gram of green weight and water loss per gram of dry weight are each 12 per cent higher for the period after spraying than for the standardization period.

TOMATO PLANTS

It has been previously stated that the Bordeaux mixture employed throughout these experiments was prepared according to the 5–5–50 formula of agricultural practice. When this mixture was applied to tomato plants, however, injury to the leaves resulted. In this experiment, therefore, the strength of the mixture was reduced to conform approximately to the 4–4–50 formula commonly used. This mixture produced no injurious effects upon the plants here employed.

In this experiment the period of standardization extended from May 26 to June 1; the period after treatment continued from June 1 to June 12. The experimental data are presented in Table VII.

TABLE VII.—*Effect of Bordeaux mixture on the rate of transpiration of potted tomato plants*

[Period before treatment, May 26 to June 1; period after treatment, June 1 to June 12, 1916]

Plant No.	Transpiration quantities.				Green weight of tops.		Dry weight of tops.	
	Period before treatment.		Period after treatment.					
	Series A.	Series B.	Series A'.	Series B.	Series A'.	Series B.	Series A'.	Series B'.
	(*A*)	(*B*)	(*'A*)	(*B*)	(*G*)	(*G'*)	(*D*)	(*D'*)
1	231.2	210.6	245.0	231.9	17.70	16.76	2.40	2.13
2	260.8	182.2	269.9	268.9	17.50	14.65	2.20	1.77
3	143.0	170.6	238.3	203.2	19.15	14.72	2.01	1.87
4	171.7	172.5	226.5	207.0	15.12	15.05	2.02	2.00
5	215.8	171.0	291.2	142.4	21.03	15.52	2.68	1.98
6	184.5	212.0	202.5	214.1	13.95	14.01	1.92	1.92
Average	201.1	186.4	245.5	211.2	17.40	15.11	2.20	1.94
Transpiration ratio	AB=1.07		A'B=1.16		Difference			0.09+
Water loss per gram of green substance ratio	AG' BG=0.93		A'G' BG=1.01		Difference			.08+
Water loss per gram of dry substance ratio	AD' BD=0.95		A'D' BD=1.02		Difference			.07+

From Table VII it will be observed that the ratios between transpiration qualities and between quantities representing water loss per gram of green substance for the periods after spraying are 8 per cent and 9 per cent higher, respectively, than the corresponding ratio values for the periods before spraying, while the ratio between quantities representing water loss per gram of dry substance is 7 per cent higher for the period after treatment. The average transpiration rate for the sprayed plants, relative to the control plants, is therefore 8 per cent higher than the average relative rate for the same plants before treating them with Bordeaux mixture.

Table VIII presents a brief summary of the average data contained in Tables III, IV, V, VI, and VII. The values of the ratios between average transpiration quantities, between average water loss per gram of green substance and between average water loss per gram of dry substance, for the periods after spraying, are here given in terms of the corresponding ratio values for the standardization periods considered as unity. The ratio values for the standardization periods are therefore omitted from the table, since all have the same relative value (1.00).

A comparison of the ratio values presented in this brief summary shows that the treated plants of each species here dealt with gave higher average rates of transpiration, relative to the rates from their respective controls, than did the same plants during the standardization periods. The lowest average rates of water loss for periods after treatment occurred with cabbage plants, showing an average increased rate of 2 per cent over the corresponding rate for the standardization period. It has already been pointed out that the low increased rates of water loss

recorded for cabbage plants may be due to the fact that the leaves were sprayed only on the upper surfaces, and that at best only a very imperfect film was obtained.

TABLE VIII.—*Average ratio values for periods after treatment, relative to the corresponding ratio values for the standardization periods taken as unity, being a summary of the average data from Tables III, IV, V, VI, and VII*

Plant.	Transpiration (ratio A':B).	Water loss per gram of dry substance (ratio A'G':BG).	Water loss per gram of green substance (ratio A'D':BD).
Cabbage	1.03	1.03	1.03
Eggplant	1.11	1.12	1.12
Pepper	1.29	1.29	1.29
Tomato	1.08	1.09	1.07
Soy bean	1.64	.....	.....

It is further to be noted that with each species employed in these experiments the three ratios, each derived from a single set of the three kinds of measurements here dealt with—transpiration, water loss per gram of green substance, and water loss per gram of dry substance—are in very close agreement. The greatest variation in the values of the three ratios occurred with tomato plants. The values of the ratios in question are 1.08, 1.09, and 1.07 for transpiration quantities, water loss per gram of green substance, and water loss per gram of dry substance, respectively. The influence of Bordeaux mixture in bringing about increased rates of transpiration varies with the different species of plants, as is indicated by the variation in the ratio values for the different species. This has already been observed in the experiments with abscised leaves.

A comparison of the results obtained from abscised leaves with those obtained from potted plants shows very clearly that the influence of Bordeaux mixture in bringing about increased rates of transpiration is much more pronounced when the spray is applied to abscised leaves than when applied to the leaves of potted plants. Thus, the ratio values representing the highest and lowest average increased rates of water loss from abscised leaves, due to an application of Bordeaux mixture, are 3.71 and 1.73, respectively, relative to the corresponding ratio values for the standardization periods taken as unity. The ratio values representing the highest and lowest average increased rates of transpiration from potted plants, following an application of Bordeaux mixture, are 1.64 and 1.02, respectively, relative to the corresponding ratio values for the time period before spraying.

SUMMARY

The results of the experiments presented above substantiate the general principle already established by Duggar and Cooley (6, 7) that the rates of transpiration from abscised leaves and also from the leaves

of potted plants are materially increased by an application of Bordeaux mixture.

A surface covering of dry, powdered copper sulphate was less effective in accelerating rates of transpiration than was a surface film of Bordeaux mixture, but was more effective than was a film of barium sulphate.

The accelerating influence of Bordeaux mixture on the rates of transpiration is much more pronounced when the spray is applied to abscised leaves than when applied to the leaves of potted plants.

The influence of Bordeaux mixture in increasing the rates of water loss from abscised leaves becomes effective immediately after the spray dries upon the leaves. The highest average increased rates of transpiration occurred during the first 2-hour period following an application of the spray.

The effectiveness of a film of Bordeaux mixture for inducing increased rates of water loss from abscised leaves varies considerably with the different species. This, to a lesser degree, is true also with potted plants.

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